THE EFFECT OF LUPIN (*LUPINUS ANGUSTIFOLIUS*) SUPPLEMENTATION ON ADAPTATION OF EWES AFTER SHORT TRANSPORT STRESS

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ABSTRACT

Vehicle transportation represents acute stress to animals with release of catecholamines and glucocorticoids from the adrenal gland resulting in impaired metabolic state. Such changes in metabolism may be reduced by the application of suitable feed supplement. The aim of this study was to test the effects of lupin supplementation applied after 1-hour transportation. Ewes in the control group (n = 7) were fed on trefoil-grass silage and hay, while the diet of the experimental group (n = 7) was supplemented with lupin groats (*Lupinus angustifolius*, var. SONET; 500 g per head per day) for 8 days. In both groups, blood was collected on the day of transportation and on Days 6 and 11 thereafter. Total blood parameters were assayed using spectrophotometry and fractions of protein, cholesterol, and lactate dehydrogenase using agarose electrophoresis. Lupin increased the albumin: globulin (ALB : GLB) ratio and beta-hydroxybutyrate (BHB) concentration and reduced serum cholesterol and lactate, however it had no effect on body weight, body condition score (BCS), plasma glucose, serum protein, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) cholesterol, lactate dehydrogenase (LDH) or alkaline phosphatise (ALP). Lupin may therefore be used as suitable feed supplement for sheep at times of high nutrient requirement.

Key words: cholesterol; glucose; lactate dehydrogenase; lupin feeding; protein; sheep; stress

INTRODUCTION

Animals react to numerous challenges during their daily routines with a variety of responses, including physical and behavioural changes in their body [24]. Short-term transportation represents acute stress to animals [31, 32] with release of catecholamines and glucocorticoids from the adrenal gland resulting in hyperglycaemia [11], reduction of live weight [37], and impaired immune system [31]. The following metabolic changes have been observed in ruminants after transport and handling stress:

1) increased activity of blood enzymes (creatine phos-

phokinase — 34, 38; lactate dehydrogenase and aspartate aminotransferase — 34);

2) increased concentrations of blood lactate [36], ketones [15], and beta-hydroxybutyric acid [18, 83]; and

3) altered blood urea nitrogen concentration [13].

Many strategies have been used for helping animals to cope with transport and handling, such as use of high-energy diets [22]. Lupin grain is a legume plant used for livestock feeding of poultry, pigs and ruminants for its good source of protein [19], which is suggested as a good potential source of plant protein very comparable in its nutritional composition to that of animal protein [41]. Moreover, lupin contains low fat and starch, lacks antinutritional substances [21, 45], and is high in non-starch polysaccharides and oligosaccharides [43]. Lupin feeding does not affect body weight, whether consumed in the short (sheep; 44) or long term (humans; 8). Lupin consumption reduces appetite and energy intake in humans [8], lowers glycaemia in rats [17], chicken [45] and humans [7]; however in cyclic female sheep it increases levels of glucose and is thus associated with higher ovulation rate [3, 44]. Lupin feeding improves blood lipids in pigs [20], rats [40] and humans [7], resulting in lower blood concentrations of cholesterol.

To test whether such effects of lupin supplementation could be applied with the same effects to ewes exposed to short-term transportation (1 hour), this study set out to evaluate metabolic blood parameters such as serum protein and its fractions (albumin and globulins and albumin: globulin ratio), cholesterol and its fractions (high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein cholesterol; HDL-C, LDL-C, VLDL-C, respectively), lactate, beta-hydroxybutyrate (BHB), activity of lactic dehydrogenase (LDH) and its isoenzymes (LDH1-5), alkaline phosphatase (ALP), and plasma glucose.

MATERIAL AND METHODS

Animals

The experiment was conducted during the sheep anoestrous period (May to June). The ewes were transported by road from a nearby sheep farm. The transport lasted 1 hour at about 20 °C and 55 % relative humidity in the morning (9.00 to 10.00 a. m.). This short-term transport caused stress to the animals. The animals were transported at an average speed of 58 km/h, avoiding abrupt accelerations and decel-erations. The experiment was carried out under standard conditions in the Experimental Station of the University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic. Fourteen Merino ewes of age 4—7 years were stabled in pens with the possibility of pasture. Water was provided to ewes ad libitum. All procedures were approved by the Ethical Committee of the State Veterinary and Food Administration of the Slovak Republic (Approval No. 2371/08-221).

Experimental Design

The ewes were divided into 2 groups: the diet of the control group (C; n = 7) consisted of trefoil-grass silage and hay, while the diet of the experimental group (L; n = 7) was supplemented with lupin groats (*Lupinus angustifolius*, var. SONET; 500 g per head per day). Sheep were fed with lupin once a day at about 5 a. m. A schematic representation of the experimental design is shown in Fig. 1. The ewes in the experimental group were fed with lupin for 8 days from Day 0 (day of transportation). The ewes were weighed on days 0 (day of transportation), 6 (fifth day of lupin supplementation), and 11 (2 days after the end of lupin feeding) and their body condition score was calculated [42].
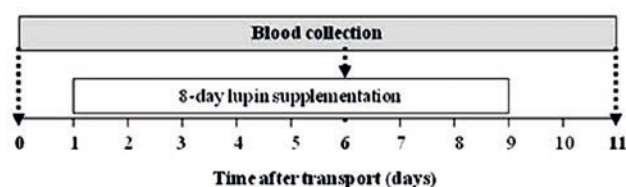

Figure 1. Schematic representation of the experimental design. Day 0 is the day of transport; Day 1 is the day of beginning of lupin supplementation which lasted 8 days (until Day 9). Blood was collected on Day 0, 6, and 11 after transport (dotted arrows)

Blood collection

Blood was collected routinely from the jugular vein of each animal with minimal disturbance to avoid excessive stress. Blood samples were collected without addition of anticoagulant on days 0 (immediately after transport), 6 and 11 of the experiment for metabolic profiles (protein, cholesterol, lactate dehydrogenase, alkaline phosphatase, beta-hydroxybutyrate, and lactate). Blood for glucose assessment was collected into centrifuge tubes with an addition of NaF. Samples were centrifuged for 10 min at 1500 × g after coagulation at room temperature (18 to 22 °C). Blood plasma for glucose was immediately assayed. Blood serum was stored at −20 °C until assayed.

Spectrophotometric assays

Assay in blood plasma

Analyses of glucose (GLUC) concentrations were performed on an ALIZÉ automatic biochemical analyzer (LISABIO, France) using commercial diagnostic kits (Randox, United Kingdom). Blood plasma was applied and replenished into substrate and absorbance was measured at 500 nm.

Assay in blood serum

In blood serum, the concentration of total cholesterol (TCH, λ 500 nm), total protein (TP, λ 540 nm), lactate (LACT, λ 500 nm), betahydroxybutyrate (BHB, λ 340 nm), activity of alkaline phosphatase (ALP, λ 405 nm) and activity of total lactate dehydrogenase (TLDH, λ 340 nm) were determined using commercial diagnostic kits (Randox, United Kingdom) with an ALIZÉ automatic biochemical analyzer (LISABIO, France).

Electrophoretic assay in blood serum

For electrophoretic study, 10 µl of serum was used for each separation. A HYDRASYS device (SEBIA, France) was used for the determination of protein and cholesterol fractions and activity of lactic dehydrogenase isoenzymes (LDH). The samples were separated using electrophoresis (HYDRAGEL 7 PROTEIN, HYDRAGEL 7 Lipo + Lp(a), HYDRAGEL 7 ISO-LDH, Ecomed, Žilina, Slovak Republic) on alkaline buffered (pH 9.2 for protein, pH 7.5 for cholesterol and pH 8.4 for LDH) agarose gels. The dried gels were prepared for visual examination and densitometry to obtain accurate relative quantification of individual zones. Then photographs of the gels were taken. Qualitative evaluations of the gels were done directly from the electropho-

retograms, and the densitometric curves of the separations were created by means of EPSON PERFECTION V 700 PHOTO densitometer scanning at 570 nm.

Statistical Analyses

The experiment was conducted in two consecutive periods due to restricted capacity for animals to be stabled at the experimental station. The conditions of the experiment were kept the same in both periods. The results were calculated for both periods together. The groups of ewes fed and not fed with lupin consisted of 7 animals in each. Since there were no statistical differences between lupin-fed and control groups in terms of metabolic parameters, the dynamic (time-relative) changes in metabolic parameters were studied separately for the control and lupin-fed group. Variances between days after transportation in ewes fed and not fed with lupin were assessed with repeated measures ANOVA with Tukey's post test (GraphPad Prism 3.0 for Windows, GraphPad Software, San Diego California USA). All data are means with S.E.M. Differences from Day 0 are marked with superscript letters and declared to be significant at levels of $P < 0.05$, $P < 0.01$.

RESULTS

Live body weight and body condition score

Neither the body condition score nor body weight of ewes were affected by the lupin feeding after transportation (Table 1).

Metabolic profiles

Total protein and electrophoretic fractions of protein in ewes fed with lupin groats after short-term transport are

Table 1. Live body weight (BW) and body condition score (BCS) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item	Day 0		Day 6		Day 11	
	Control	**Lupin**	**Control**	**Lupin**	**Control**	**Lupin**
BW [kg]	40.36 ± 2.07	43.43 ± 1.46	42.71 ± 1.96	45.29 ± 1.61	39.27 ± 2.32	43.26 ± 1.82
BCS	2.43 ± 0.19	2.82 ± 0.11	2.50 ± 0.16	2.82 ± 0.11	2.50 ± 0.16	2.82 ± 0.11

Table 2. Mean ± SEM concentrations of total protein (TP), albumin (ALB), alpha 1-globulin (alpha-1 GLB), alpha 2-globulin (alpha-2 GLB), beta globulin (beta GLB), gama globulin (gama GLB), and albumin: globulin ratio (ALB: GLB ratio) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [g.l⁻¹]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TP	66.43 ± 7.10	72.42 ± 3.46	68.63 ± 3.62	72.13 ± 3.90	70.93 ± 3.83	69.50 ± 2.44
ALB	23.63 ± 2.64	33.18 ± 1.81	23.83 ± 3.55	35.27 ± 1.48	23.86 ± 4.03	34.73 ± 1.37
Alpha-1 GLB	5.78 ± 1.55	4.75 ± 0.37	7.22 ± 1.67	4.23 ± 0.44	6.86 ± 1.62	3.87 ± 0.15
Alpha-2 GLB	8.85 ± 1.71	8.40 ± 0.56	9.61 ± 1.36	7.86 ± 0.28	9.81 ± 1.55	7.18 ± 0.20
Beta GLB	12.00 ± 5.95	7.19 ± 1.70	9.94 ± 3.42	4.95 ± 1.16	10.07 ± 3.34	4.13 ± 0.61
Gama GLB	16.18 ± 1.92	18.90 ± 1.19	18.15 ± 4.58	19.83 ± 1.98	20.34 ± 5.11	19.69 ± 1.76
ALB: GLB ratio	0.66 ± 0.13	0.88 ± 0.10[a]	0.58 ± 0.12	1.00 ± 0.10[a]	0.56 ± 0.14	1.03 ± 0.10[a]

[a] — $P < 0.05$; values within rows, Day 6 and/or Day 11 compared to Day 0

Table 3. Mean ± SEM concentrations of total cholesterol (TCH), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C) cholesterol of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [mmol.l⁻¹]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TCH	1.74 ± 0.23	1.93 ± 0.24[a]	1.52 ± 0.13	1.71 ± 0.22[a]	1.05 ± 0.43	1.68 ± 0.21[a]
HDL	0.18 ± 0.03	0.19 ± 0.07	0.12 ± 0.03	0.21 ± 0.03	0.14 ± 0.03	0.29 ± 0.04
LDL	0.86 ± 0.10	0.96 ± 0.18	0.83 ± 0.12	0.78 ± 0.12	1.01 ± 0.20	0.67 ± 0.12
VLDL	0.79 ± 0.11	0.70 ± 0.10	0.57 ± 0.06	0.72 ± 0.10	0.68 ± 0.11	0.72 ± 0.09

[a] — $P < 0.01$; values within rows, Day 6 and/or Day 11 compared to Day 0

shown in Table 2. Lupin supplementation did not affect the concentration of total protein, albumin, and globulin fractions after short-term transport of ewes. However, the albumin: globulin (ALB:GLB) ratio was significantly increased (P < 0.05) during lupin supplementation and 2 days after lupin withdrawal (Day 11). In the control group, the protein parameters were not affected after transport stress.

Total cholesterol and its fractions in ewes fed and not fed with lupin groats immediately after transportation are shown in Table 3. Total cholesterol was significantly lowered (P < 0.01) by the lupin in ewes 6 days after transport, but its fractions were not significantly affected. Total cholesterol and its fractions in the control group were not affected after transport.

The activity of total lactate dehydrogenase (TLDH) in blood serum and LDH isoenzymes in blood plasma of ewes fed and not fed with lupin groats for 8 days were not affected after short transport (Table 4). The activity of alkaline phosphatase (ALP) in blood serum was not affected either in the control or in the lupin-fed group after short transport stress.

Serum concentrations of beta-hydroxybutyrate (BHB), lactate and plasma glucose of ewes fed and not fed with lupin groats for 8 days after transport stress are shown in Table 5. Serum BHB concentration in lupin-fed ewes significantly decreased 2 days after lupin withdrawal (Day 11). There was hyperglycaemia in both groups after transport. Plasma glucose concentrations significantly decreased after transport

Table 4. Mean ± SEM activity of total lactate dehydrogenase (TLDH), LDH isoenzymes (1—5), and alkaline phosphatase (ALP) of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [μkat.l⁻¹]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
TLDH	14.97 ± 1.54	17.62 ± 1.21	15.22 ± 0.95	15.38 ± 0.90	15.62 ± 1.23	15.10 ± 0.88
LDH1	6.54 ± 0.86	9.43 ± 0.80	7.30 ± 0.49	8.47 ± 0.48	7.43 ± 0.39	7.92 ± 0.23
LDH2	1.20 ± 0.22	1.11 ± 0.05	1.08 ± 0.15	0.92 ± 0.07	1.17 ± 0.16	1.00 ± 0.08
LDH3	4.72 ± 0.55	4.49 ± 0.54	4.57 ± 0.40	3.82 ± 0.33	4.34 ± 0.34	3.74 ± 0.39
LDH4	0.98 ± 0.27	1.18 ± 0.19	1.38 ± 0.51	1.04 ± 0.05	1.64 ± 0.76	1.11 ± 0.07
LDH5	1.52 ± 0.45	1.41 ± 0.38	0.90 ± 0.17	1.14 ± 0.14	1.04 ± 0.13	1.34 ± 0.21
ALP	1.57 ± 0.18	1.53 ± 0.19	1.56 ± 0.14	1.85 ± 0.32	1.55 ± 0.12	1.98 ± 0.14

Table 5. Mean ± SEM concentrations of beta-hydroxybutyrate (BHB), glucose, and lactate of ewes fed and not fed with lupin groats for 8 days after transport stress

Item [mmol.l⁻¹]	Day 0		Day 6		Day 11	
	Control	Lupin	Control	Lupin	Control	Lupin
BHB	0.24 ± 0.02	0.24 ± 0.04	0.33 ± 0.02	0.36 ± 0.04 [a]	0.31 ± 0.06	0.22 ± 0.03 [a]
Glucose	6.54 ± 0.89 [a, b]	7.94 ± 1.66 [a]	3.86 ± 0.38 [b]	4.13 ± 0.17 [a]	3.94 ± 0.30 [a]	3.82 ± 0.18 [a]
Lactate	5.32 ± 1.12	7.12 ± 0.98 [a, b]	4.29 ± 0.69	3.20 ± 0.65 [a]	2.55 ± 0.52	2.26 ± 0.31 [b]

[a] — P < 0.05; [b] — P < 0.01; values within rows, Day 6 and/or Day 11 compared to Day 0

in both groups (P < 0.05 and P < 0.01 in control; P < 0.05 in lupin-fed), but there was no significant difference between the groups. Similarly, serum lactate concentration decreased significantly after transport, both during lupin feeding (P < 0.05) and 2 days after lupin withdrawal (P < 0.01).

DISCUSSION

Short-term lupin feeding showed no significant effect on body weight and body condition score after 1-h transport stress, which is consistent with the observations of V i ñ o l e s et al. [44], who observed similar results in ewes fed with lupin for short time, but not after transportation. K a d i m et al. [10] reported that 2-h transportation decreased body weight in lambs due to dehydration and food deprivation.

In our study, lupin feeding for 8 days after 1-hour transport did not affect the serum concentration of total protein and/or its fractions, but significantly increased the albumin : globulin ratio. The ewes had free access to water, so the ALB : GLB ratio could not have been increased due to dehydration when albumin was increased [12], but to decreased globulin fraction (alpha and beta globulin). This decrease in alpha and beta globulin fractions could be associated with the reduction of serum cholesterol concentration as observed in birds fed on lupin diets [47]. The finding of no effect of lupin on protein parameters is consistent with the observations in lupin-fed pigs [48].

Total cholesterol concentrations were significantly decreased by lupin supplementation in this study, which corroborates the widely-known cholesterol-lowering effect of lupin [7, 9, 20, 40]. The reduction of serum cholesterol results from the effect of lupin fibre [7, 9, 30]. The cholester-

ol-lowering effect of lupin is explained by several theories, including the effect of amino acid composition of dietary protein [6], lower absorption of cholesterol in the small intestine of piglets [20] and hamsters affected probably by lupin phytosterols, and/or it can also be associated with the stimulation of low-density lipoprotein (LDL) receptors by the protein component of lupin seeds [29, 39]. Some studies have reported reduction of LDL [18, 27] and VLDL cholesterol concentrations and tendency of HDL cholesterol to increase in rats [39], which is consistent with our results, although we found no change in VLDL concentration.

The present study showed no effect of lupin feeding on the activity of lactate dehydrogenase and alkaline phosphatase in the blood serum of ewes after transport. These observations are inconsistent with some studies (ALP – 34, 38; LDH – 34) which showed clear impact of transport on the activity of these enzymes, even though the animals were subjected to transport stress for a longer time, and lupin supplementation slightly decreased the activity of LDH [27] in hypercholesterolemic rats. Isoenzyme LDH-5 is an important diagnostic marker of transport stress [35], because it is used mostly by the cells of the liver and skeletal muscle and is released into the blood stream during stress. We suggest that maybe a higher dose of lupin grain can be used to improve the function of the liver and skeletal muscles impaired by transport stress, but this suggestion needs further confirmation. The metabolism of glycogen plays a key role during the muscle-to-meat transition. Lactate dehydrogenase enzyme participates in the metabolism of glycids by catalysing the reversible conversion of pyruvic acid to lactic acid. Lactic acid level reflects quantitative transformation of glycogen and indicates typical or atypical processes of meat ripening [14]. Perimortal situations increasing energy metabolism have considerable effects on factors that influence the ripening of meat. Meat of transported animals, e.g. wild rabbits, has lower concentrations of lactic acid, higher concentrations of phosphates and higher pH than for example hunted wild rabbits [16].

The mobilization of body fat reserves and energy balance can be efficiently determined by measuring serum concentrations of betahydroxybutyrate (BHB) [33] when nutritional stress is indicated and concentrations are over 0.8 mmol.l^{-1}. The present study showed serum BHB to be in the physiological range (0.34—0.68 mmol.l^{-1}); however the concentration of BHB significantly decreased after lupin withdrawal. This could mean that lupin supports produc-

tion of ketones, but this theory should be further investigated. These observations are inconsistent with previous studies [46] where these concentrations were increased after transport. Lactate concentrations after transport were in the physiological range in both groups, control and lupin-fed. These observations are inconsistent with Mitchell et al. [23] and Schaefer et al. [36], who reported increases in serum lactate concentrations after transport stress. We suggest that the significant decrease in serum lactate as a result of lupin supplementation after transport may support the utilization of lactate for gluconeogenesis in ruminants after transport stress.

In the present study, plasma glucose concentrations in control and lupin-fed ewes reflected the hyperglycaemic effect [1] of increased activity of stress hormones [11], reaching a maximum after 2 hours from loading onto the transport vehicle [2], primarily due to breakdown of glycogen in the liver [25]. There was no difference between the groups, however, so we suggest that lupin had no effect on glucose concentrations after transport. Our findings are in contrast to previous studies which reported that lupin supplementation decreased blood glucose in rats [27], reflecting the effect of dietary fibre from the legumes [28] and saponins [26] which have hypoglycaemic activity, although it increased plasma glucose in ruminants. In goats, plasma glucose concentrations increased and reached the maximum 2 hours after 2.5-h transport and then decreased to pre-loading values.

We conclude that lupin has a cholesterol-lowering effect also when consumed in the short term. Lupin increased the ALB: GLB ratio and BHB concentration and reduced serum lactate, however it had no effect on body weight, BCS, plasma glucose, serum protein, HDL-C, LDL-C, VLDL-C, or ALP. Lupin may therefore be used as a suitable feed supplement for sheep at times of high nutrient requirement.

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