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Residual feed intake and hematological and metabolic blood profiles of Ile de France lambs

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ABSTRACT - The objectives of this study were to estimate the phenotypic correlations of residual feed intake (RFI) and gross feed efficiency (GFE) with hematological and metabolic blood profiles of lambs and to determine the differences for these traits in animals of different RFI classes. Twenty Ile de France male lambs, 115±8 days of age and 31.3±4.1 kg of body weight (means ± SD), were individually housed and their dry matter intake was measured over 65 days. They were weighed every 13 days to determine the average daily weight gain and two blood samples were collected at the last two weighings (at 07h30) for analysis of blood variables. The animals were divided into two classes: negative RFI (most efficient: <0.5 SD below the mean; n = 6) and positive RFI (least efficient: >0.5 SD above the mean; n = 8). There were associations among RFI and the serum metabolic variables for albumin (r_{RFI} = 0.74) and creatinine (r_{RFI} = −0.45) and between GFE and serum albumin (r_{GFE} = −0.70). Less efficient animals as measured by RFI had higher serum albumin and lower creatinine levels and showed a tendency to have a greater concentration of total plasma protein. Other serum biochemical parameters were not correlated with GFE and RFI, and no differences between RFI classes were found. There was a correlation between the percentage of eosinophils and RFI (r_{RFI} = −0.65), and such more efficient animals had a higher proportion of these cells and a trend to have a lower percentage of monocytes. This study provided evidence indicating associations between RFI and protein metabolism, as reflected by the serum albumin and creatinine. The hematological findings suggest that RFI is related to susceptibility of lambs to stress and should provide a basis for further research in this regard.

Key Words: blood biochemistry, feed efficiency, hematology, metabolism, sheep

Introduction

Feed is often one of the most expensive items in sheep breeding cost analyses (Barros et al., 2009). The feed efficiency of animals has an important influence on the profitability of production systems and provides the potential to identify and select animals with an improved ability to convert feed into animal product. Improved efficiency of livestock also plays an important role in reducing their environmental impact because the grazing areas needed for livestock can be reduced, and waste production, such as manure and methane, may decrease with a better use of diet (Basarab et al., 2003).

Gross feed efficiency (GFE) can be interpreted as the effectiveness of converting ingested feed into products. Among other measures that have emerged over the years for the evaluation of animal feed efficiency, residual feed intake (RFI) was proposed for beef cattle mainly because it is not directly correlated with the rate of gain and body weight (BW), which is important because these latter traits can affect feed requirements (Arthur et al., 2001). Residual feed intake is defined as the difference between the observed feed consumption and the estimated feed consumption of an animal, thus the more negative the residual portion is, the greater the feed efficiency of an animal.

Due to the high costs of the precise determination of individual feed intake in sheep and cattle, which would limit the adoption of feed efficiency as a tool to aid in the genetic selection on a large scale (Lanna & Almeida, 2004), the search for physiological parameters such as blood indicators predictive of RFI becomes useful as a means for early indirect selection in large herds. There is also interest in better understanding the possible physiological variation in the efficiency of diet use among individuals. The blood profile has also been studied because it is related, along with other factors, to the stress to which the animals are subjected (Hickey et al., 2003). According to Richardson & Herd (2004), among the physiological processes that

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regulate RFI variation among individuals, 37% of them can be attributed to metabolism and stress. For sheep, there are few studies on feed efficiency from an RFI perspective (Knott et al., 2010; Redden et al., 2011; Rincon-Delgado et al., 2011). Inferences about the subject are supported primarily by information from other species, especially cattle.

The objectives of this study were to estimate the phenotypic correlations between feed efficiency, represented by GFE and RFI, and the hematological and blood biochemical parameters of lambs, and to investigate the differences in blood variables in animals classified according to RFI.

Material and Methods

This research project was approved (protocol number 010/2011) by the Animal Use Ethics Committee of the Agricultural Sciences Campus of Universidade Federal do Paraná (Federal University of the State of Paraná, Southern Brazil). The animals used in this study were individually housed in elevated pens with a slatted floor (approximately 1.0 × 2.2 m) equipped with feeders and a water source. Twenty non-castrated Ile de France male lambs with 115±8 days of initial age, 31.3±4.1 kg of initial BW and 51.8±4.2 kg of final BW (means ± SD), offspring of same ram were used, from the Tangara farm in Reserva - PR (kept on pasture).

After 17 days of adaptation to diet and management, the animals were kept confined for 65 days and individual dry matter intake (DMI) was measured. Feed was offered *ad libitum* and provided three times a day, at 8h00, 13h00 and 17h00, in the form of total mixed ration (Table 1) with a forage:concentrate ratio of 30:70. The individual feed amount was adjusted daily to ensure a 10% refusal based on the DMI from the previous day. The mean chemical composition of the feed (Table 1) was obtained through daily sampling and the formation of weekly composite samples. The levels of neutral and acid detergent fiber were determined as described by Van Soest et al. (1991), while other nutrients were determined according to the methodologies described by AOAC (1995) and the total digestible nutrients were calculated as reported by Weiss et al. (1992). The diet had reached the minimum value of 2.4 Mcal ME/kg DM stipulated to not limit the feed intake at consumption tests in cattle, according to the Beef Improvement Federation (BIF, 2010). Feed refusals were also weighed and sampled each morning, allowing the formation of weekly composite samples for the determination of DM and later to obtain observed dry matter intake (DMI_{obs}), which was calculated as the difference between the amount of feed offered and the feed refused. The average DM content of the feed refused was 76.2%.

The animals were treated for gastrointestinal parasites using Cydectin® and the dose followed the recommendation of the manufacturer during the adaptation period and the gastrointestinal parasite burdens were monitored every 14 days by fecal egg and oocyst counts (Gordon & Whitlock, 1939).

During the experimental period, lambs were weighed every 13 days and the average daily weight gain (ADG) was determined as the angular coefficient by regressing the individual BW against the experimental time. The gross feed efficiency (GFE) was calculated as the ratio between the ADG and the DMI_{obs}. The residual feed intake (RFI) was measured as the difference between the DMI_{obs} and the estimated dry matter intake (DMI_{est}) (Koch et al., 1963). The DMI_{est} was calculated by regressing the daily DMI_{obs} as a function of metabolic BW (midtest weight$^{0.75}$) and ADG for each animal during the experiment, using R Development Core Team statistical software (2010), following the model: $DMI_{est} = \beta_0 + \beta_1 BW_{met} + \beta_2 ADG + \varepsilon$. After calculating the RFI coefficient for each animal, the lambs were classified into the highest positive (least efficient: >0.5*standard deviation above the mean; n = 8) and lowest negative (most efficient: <0.5*standard deviation below the mean; n = 6) efficiency classes based on RFI.

At the last two weighings, two blood samples were collected (7h30) from each animal via jugular vein puncture with vacuum tubes using a 25 gauge 8 mm needle.

Metabolic measurements were conducted in the blood serum. Blood samples were collected in tubes

Table 1 - Ingredients and chemical composition of total mixed diet given to lambs

Ingredient	Inclusion (g/kg)
Chopped ryegrass hay	300.00
Limestone	11.90
Soybean hulls	140.00
Soybean meal 44%	116.90
Milled corn	408.10
Mineral mix[1]	23.10
Sodium monensin	0.024
Chemical composition	% (DM)
Dry matter	87.70
Crude protein	14.12
Ether extract	2.87
Ash	6.32
Acid detergent fiber	18.14
Neutral detergent fiber	34.63
Total digestible nutrients	66.56
Calcium	1.13
Phosphorus	0.47

[1] Ovinofós Núcleo Produção® - Tortuga Companhia Zootécnica Agrária – São Paulo, SP, Brazil. Guaranteed levels (per kg of product): P - 61 g; Ca - 267 g; S - 35 g; Co - 20 mg; Mn - 2,000 mg; Cu - 350 mg; F - 610 mg; Se - 23 mg; Mg - 20 g; Cr - 60 mg; Mo - 500 mg; Zn - 6,000 mg; Fe - 3,000 mg; I - 80 mg.

without anticoagulant and remained at rest until complete coagulation. The samples were then centrifuged at 1800 x g for 10 minutes. The serum was removed and stored in micro tubes at −20 ºC until analysis. Using a Mindray BS-200 automatic biochemical analyzer and commercial kits (Katal Biotecnológica Indústria e Comércio Ltda, Belo Horizonte, MG, Brazil), the serum metabolite concentrations were determined (in accordance with the manufacturers guidelines): albumin (colorimetric method of bromocresol green), aspartate transaminase enzyme (ultraviolet kinetic method of aminotransferase aspartate), direct bilirubin and total bilirubin (colorimetric method of Sims-Horn), creatine phosphokinase (ultraviolet kinetic method of creatine phosphokinase), cholesterol (enzymatic colorimetric method of cholesterol oxidase-peroxidase-4-aminophenazone), creatinine (Jaffé colorimetric method), gamma-glutamyl transferase (ultraviolet kinetic method of gamma-glutamyl transferase), glucose (enzymatic colorimetric method of glucose), total proteins (colorimetric method of biuret), triglycerides (enzymatic colorimetric method of glycerophosphate oxidase-peroxidase-4-aminophenazone) and urea (urease enzymatic colorimetric method). Serum globulin values were obtained by subtracting the serum albumin values from the total protein. The albumin:globulin ratio was calculated by dividing the albumin fraction value by the globulin fraction value of each sample.

The hematologic measurements were conducted using blood plasma. Blood plasma samples were collected in vacuum tubes containing anticoagulant (Ethylenediamine tetraacetic acid - EDTA) and slides with blood smears were prepared for analysis of the white blood cell count. Differentiated cells that were counted and expressed as a percentage of the leukocytes were eosinophils, basophils, monocytes, lymphocytes and neutrophils, allowing later determination of the neutrophil/lymphocyte ratio. In addition, the percentage for hematocrits was determined using the microhematocrit technique (Farrand, 1976) and the automated count of erythrocytes, total leukocytes and hemoglobin was performed using an auto hematology analyzer (BC 2800 Vet, Myndray). The mean globular volume (MGV) was calculated using the formula: (hematocrit × 10)/total of erythrocytes. The mean globular hemoglobin (MGH) was calculated using the formula: (hemoglobin × 10)/total erythrocytes; and the mean globular hemoglobin concentration (MGHC) was calculated using the formula: (hemoglobin/hematocrit × 100) as previously reported by Wintrobe (1933). The fibrinogen concentration was estimated by heat precipitation in relation to the concentration of total plasma protein determined by refractometry (Thomas, 2000).

The residual feed intake correlations with the mean values of blood variables were estimated (n = 20) by Pearson's simple correlation analysis using the cor.test analytical procedure of the R Development Core Team (2010) statistical program. The data from different efficiency groups were subjected to analysis of variance to verify the difference by an F test at 5% significance. To examine the associations with the degree of parasitic infection, the Spearman correlation was adopted, since this data showed no normal distribution ($P<0.05$, by Shapiro-Wilk test).

Results

The formula resulting from the estimation of DMI and its coefficient of determination (R^2) for this experiment was:

$$DMI_{est} = 0.09830 + 0.07470*BW_{met} + 0.62617*ADG$$
$$(R^2 = 0.34)$$

The animals consumed a mean ± SD of 1.53±0.16 kg of ration (DM) per day during the study period, which is equivalent to approximately 3.7±0.34% of BW (Table 2). These values were close to those reported by NRC (2006) for lambs in the weight and age ranges used in the experiment.

The minimum and maximum values for RFI were respectively -0.426 kg DM/day and 0.149 kg DM/day, i.e., the least efficient animal consumed approximately 0.575 kg DM/day more feed than the most efficient, showing there is wide variation in consumption among individuals, which can also be seen in the standard deviation for the RFI, which was 0.129 kg DM/day. Among the RFI classes (Table 2), the mean difference was 0.240 kg DM/day ($P<0.01$). The low RFI group showed a tendency to be also more efficient by GFE ($P = 0.10$) and had a lower DMI_{obs} ($P = 0.02$) and lower DMI in BW percentage ($P<0.01$), with a similar ADG ($P>0.10$) in the test period.

Both the parameters for direct bilirubin and the basophils expressed as a percentage of the leukocytes had values equal to zero in all of the blood samples. The serum albumin concentration was significantly correlated with RFI ($P<0.01$) and GFE ($P<0.01$), with correlation coefficients of 0.74 and −0.70, respectively (Table 3).

In addition to the moderate correlation between RFI and this blood parameter, a significant difference ($P = 0.04$) among the RFI efficiency classes (Table 3) was detected in the serum albumin levels, with the most efficient animals showing the lowest concentrations. There was a negative correlation ($P<0.05$) between RFI and the mean concentration of serum creatinine ($r_{RFI} = −0.45$) and a comparison of the two RFI groups showed significant differences for this variable ($P<0.01$). The high-RFI lambs

tended to have a greater concentration of total plasma protein than the lambs with a low RFI (P = 0.08).

There were no statistically significant phenotypic correlations between GFE and the studied hematological components (Table 4). Similarly, RFI was not correlated with these blood parameters, except for the percentage of eosinophils (P<0.01) that had a moderate negative correlation (r_{RFI} = −0.65).

Table 2 - Overall means (n = 20), means and standard error of the mean (SEM) obtained for observed dry matter intake (DMIobs), dry matter intake as a percentage of body weight (DMI %BW), average daily gain (ADG), gross feed efficiency (GFE) and residual feed intake (RFI) of lambs ranked as either high or low for RFI

Item	Means	RFI (classes)				
		High (n = 8)	SEM	Low (n = 6)	SEM	P
DMIobs (kg/day)	1.527	1.633	0.042	1.422	0.069	0.020
DMI (%BW)	3.69	3.94	0.04	3.31	0.15	0.001
ADG (kg/day)	0.332	0.335	0.014	0.329	0.013	0.760
GFE	0.219	0.206	0.009	0.235	0.016	0.100
RFI (kg/day)	0.000	0.103	0.011	−0.137	0.060	0.001

P>0.05 means that there was no significant differences by the F test.

Table 3 - Pearson's correlation coefficients (n = 20) of blood biochemical components with residual feed intake (r_{RFI}) and gross feed efficiency (r_{GFE}) and means and standard error of the mean (SEM) obtained for these blood metabolites for lambs ranked as high or low for RFI

Item	Correlations		RFI (classes)				
	r_{RFI}	r_{GFE}	High (n = 8)	SEM	Low (n = 6)	SEM	P
Albumin (g/dL)	0.74**	−0.70**	3.62	0.03	3.51	0.04	0.040
AST (U/L)	0.03	−0.22	93.04	4.33	89.40	3.92	0.560
Total bilirubin (mg/dL)	−0.25	0.14	0.38	0.03	0.41	0.02	0.370
CK (U/L)	0.17	−0.27	170.79	13.04	166.60	19.84	0.860
Cholesterol (mg/dL)	0.09	−0.22	53.36	2.22	53.46	2.18	0.970
Creatinine (mg/dL)	−0.45*	0.24	0.74	0.03	0.86	0.02	0.006
GGT (U/L)	0.04	−0.01	52.36	4.08	52.15	3.46	0.979
Glucose (mg/dL)	0.05	0.35	81.91	1.29	81.68	1.75	0.919
Globulin (g/dL)	0.06	−0.22	2.62	0.11	2.45	0.08	0.259
Total protein (g/dL)	0.30	−0.42	6.24	0.11	5.96	0.07	0.079
Albumin:globulin	0.14	0.02	1.40	0.06	1.44	0.05	0.619
Triglycerides (mg/dL)	0.13	0.05	19.14	0.96	18.92	1.23	0.899
Urea (mg/dL)	−0.27	0.15	45.34	2.36	47.64	2.14	0.509

AST - aspartate transaminase; CK - creatine phosphokinase; GGT - gamma glutamyltransferase.
*P<0.05 and **P<0.01.
P>0.05 means that there was no significant differences by the F test.

Table 4 - Pearson's correlation coefficients (n = 20) of hematological components and fibrinogen concentration with residual feed intake (r_{RFI}) and gross feed efficiency (r_{GFE}) and means and standard error of the mean (SEM) obtained for these traits for lambs ranked as either high or low for RFI

Item	Correlations		RFI (classes)				
	r_{RFI}	r_{GFE}	High (n = 8)	SEM	Low (n = 6)	SEM	P
MHC (%)	0.11	−0.03	30.07	0.28	29.05	0.57	0.110
Erythrocytes (106/uL)	0.06	−0.15	11.75	0.27	11.43	0.61	0.610
Fibrinogen (g/dL)	0.09	−0.26	0.23	0.03	0.30	0.07	0.290
Hematocrit (%)	−0.04	−0.33	35.81	0.27	35.92	0.93	0.900
MGH (pg)	0.03	−0.12	9.20	0.24	9.18	0.22	0.950
Hemoglobin (g/dL)	0.12	−0.36	10.77	0.11	10.43	0.33	0.300
Mean globular volume (fL)	−0.04	−0.09	30.61	0.83	31.70	1.17	0.450
Leukocytes (103/uL)	0.18	−0.02	9.14	0.53	8.13	0.761	0.280
Lymphocytes (%)	0.17	−0.30	52.19	2.67	50.25	3.81	0.670
Eosinophils (%)	−0.65**	0.27	2.75	0.41	6.08	0.83	0.002
Monocytes (%)	0.33	−0.48	5.63	1.66	2.25	0.31	0.098
Neutrophils (%)	−0.14	0.41	39.44	2.85	41.42	3.84	0.670
Neutrophils:lymphocytes	−0.17	0.39	0.78	0.09	0.88	0.15	0.560

MHC - mean hemoglobin concentration; MGH - mean globular hemoglobin.
*P<0.05 and **P<0.01.
P>0.05 means that there was no significant differences by the F test.

Likewise, the RFI classes differed only for eosinophils, with the most efficient animals having a higher proportion of eosinophils in the leukocyte count (P<0.01). The percentage of monocytes showed a trend towards significance, with the high RFI class showing a larger number of these cells than the low RFI class (P<0.10).

Discussion

There was considerable variation in feed efficiency using the RFI approach between lambs, with the most efficient animals having lower dry matter intake (12.92% lower in the low RFI class). Therefore, RFI could become an important tool to increase profitability in the meat production industry. François et al. (2002) and Snowder &Van Vleck (2003) reported a moderate heritability for RFI in sheep of 0.30 and 0.26, respectively, which indicates the possibility of response to individual selection. However, it is essential to know the effects of using RFI as a genetic selection criterion in sheep.

Albumin is the most abundant protein in the blood and accounts for 50 to 65% of the total blood protein content (Contreras et al., 2000). It is also an important constituent of protein metabolism and a relevant indicator of the protein nutritional status (Agenas et al., 2006). The occurrence of relationships between serum albumin and the efficiency measures can be linked to a greater DMI by the less efficient animals, as described by Connell et al. (1997), who showed that serum albumin levels are closely associated with the level of feed intake and nutrient supply.

Most cattle studies reporting effects of RFI on blood concentrations of serum creatinine suggest that the RFI-efficient cattle had a higher concentration (Santana, 2009), similar to what occurred with the lambs in this study. Serum creatinine is an indicator of protein metabolism in ruminants and is positively correlated with muscle mass (Cameron, 1992; Caldeira et al., 2007) and negatively correlated with backfat thickness in sheep (Clarke et al., 1996). In this context, it is suggested that animal selection for RFI may result in higher concentrations of blood creatinine and, concurrently, lead to changes in body composition with lower fat deposition in the carcass and a higher proportion of muscle mass similar to that found in cattle by Richardson et al. (2001). Therefore, it is important that future studies in sheep, as have been undertaken in cattle, be conducted to evaluate possible changes in animal body composition and carcass traits (Kelly et al., 2010; Schaffer et al., 2011).

Richardson et al. (2004) estimated similar negative correlation (r = –0.45) between serum creatinine and RFI for beef steers and found significant positive correlations among RFI and blood glucose level (r = 0.40) and aspartate transaminase enzyme (r = 0.43). However, in this study, with exception of albumin and creatinine, the blood biochemical components examined were not correlated with either RFI or GFE, and in relation to the other metabolites, the efficiency classes were similar in blood biochemical profile (Table 3), unlike what was described by Rincon-Delgado et al. (2011), who found the most efficient sheep and rams exhibiting lower glucose levels. In cattle, the high-RFI class animals had greater total plasma protein levels (Richardson et al., 2004), corroborating the trend shown in this study.

In the case of hematological components, Rincon-Delgado et al. (2011) reported lower values of total erythrocytes and total leukocytes as well as higher mean globular volume and mean corpuscular hemoglobin for the most RFI-efficient rams. They also described higher mean corpuscular hemoglobin for the most RFI-efficient ewes compared with the least efficient ones. However, this behavior was not observed for the lambs in this study, possibly because the animals were younger.

The results available in the literature indicate that high-RFI steers are more susceptible to stress-inducing conditions (Richardson et al., 2002). According to Richardson & Herd (2004), the stress causes glucocorticoid release in cattle, which leads to changes in the pattern of white blood cell count. Additionally, Knott et al. (2010) reported higher serum cortisol concentrations in less RFI-efficient rams. The rise in cortisol production resulting from acute stress can result in neutrophilia, lymphopenia, monocytosis and eosinopenia (Jain, 1993). Thus, the lower number of eosinophils in the blood of less efficient lambs and the tendency observed in relation to the percentage of monocytes in the current study may be some of the typical indicators of a stress leukogram in ruminants (Jones & Alison, 2007).

Changes in the sheep leukogram are often associated with parasitosis. Nevertheless, since the experiment occurred in confinement with good hygiene conditions and the eimeriosis prevention was made with sodium monensin in the diet, the verminosis was not an expressive factor and the percentage of eosinophils was not correlated with the degree of parasitic infection in the lambs in any of the assessments herein and neither were the mean values (P>0.10). This finding demonstrates the need for additional studies to confirm the percentage of eosinophils as a potential indicator of feed efficiency and especially to verify the occurrence of additional and complementary effects related to stress and to RFI differences in these animals.

Conclusions

Lambs with low residual feed intake have similar average daily gain and consume less feed than the high-residual feed intake lambs, which shows that this trait can be used as a tool to identify more efficient feedlot lambs. This study provided evidence indicating associations between residual feed intake and protein metabolism as reflected mainly by serum albumin and creatinine. The hematological findings suggest the relationship of residual feed intake with stress susceptibility and should provide a basis for further research to better characterize the relationship between such factors and to identify physiological indicators that could be predictive of residual feed intake in sheep.

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