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Relationship of Residual Feed Intake on Specific Hematological and Biochemical Parameters in Rambouillet Sheep

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Abstract: Relationships of Residual Feed Intake (RFI) on hematological and biochemical parameters were determined in Rambouillet sheep. After feeding period of 42 days (research period), RFI was calculated in 16 rams and 24 ewes. Lowest negative and highest positive RFI (<0.5 SD below the mean and >0.5 SD above the mean, respectively) rams (n = 8) and ewes (n = 12) were selected. From RFI-categorized sheep, hematological and biochemical parameters were evaluated at day 0, 21 and 42 and at day 0 and 42 of the feeding period, respectively. Positive RFI ewes (less efficient) tended ($p \leq 0.10$) to have more red blood cells (RBC, 10^6 μL) (9.66 vs. 7.68) and less Mean Corpuscular Hemoglobin (MCH, pg) (9.59 vs. 12.95) than negative RFI ewes. Positive RFI rams have ($p < 0.05$) more RBC (9.27 vs. 6.67) and White Blood Cells (WBC, 10^3 μL) (10.25 vs 7.77), less Mean Corpuscular Volume (MCV; 30.98 vs. 44.11 fL) and tended ($p < 0.10$) to have lesser MCH (11.50 vs. 16.12) than negative rams. Glucose (mg dL^{-1}) increased ($p \leq 0.01$) in positive RFI ewes (77.30 vs. 63.57) and rams (80.23 vs. 51.44) in comparison with more efficient ruminants. Data obtained suggest that positive RFI sheep could be more susceptible to stress compared to more efficient sheep.

Key words: Erytrocyte, glucose, leucocyte, residual feed intake, triglyceride, RFI

INTRODUCTION

Profitability of production can be asses by improvements in feed efficiency, lowering input costs and undesired environmental impacts. In most sheep operations, feed is the largest single expense (Cammack *et al*., 2005). Residual Feed Intake (RFI) is a measure of feed efficiency in growing animals allowing comparisons between individuals which differ in level of production during the measurement period. By definition, RFI is the difference between an animal's actual feed intake and its expected feed intake on the basis of size and growth (Koch *et al*., 1963).

It is independent of the level of production being negative-RFI the most efficient. RFI represents inherent variation in basic metabolic processes which are related to efficiency (Herd and Bishop, 2000). Even so automatic feed intake recorders are now available, the cost and technical difficulties in measuring RFI has been restricted its adoption. Measurement of hematological and biochemical parameters can provide information about health, level of production, feed efficiency and stress of the animals during different physiological stages (Azab and Maksoud, 1999; Jones and Allison, 2007; Polizopoulou, 2010; Russell and Roussel, 2007; Shaw and Tume, 1992). Red Blood Cells (RBC) are produced in the bone marrow in response to erythropoietin produced primarily by the kidneys. They are responsible for gas exchange, carrying oxygen and carbon dioxide in their heme structures. The Mean Corpuscular Volume (MCV), mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) are characteristics of the RBC, indicating average cell size, average cell Hemoglobin (Hgb) content and average cell Hgb concentration, respectively.

White Blood Cells (WBC) including granulocytes and mononuclear cells are produced and matured in the bone marrow with the exception of lymphocytes which are originating from a bone marrow stem cell but undergoing maturation and proliferation in other lymphoid tissues. Two major protein components of total serum proteins are albumin and globulin. Albumin is synthesized in the liver

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and is responsible for the oncotic pressure in plasma. A large portion of the globulin fraction consists of immunoglobulins which are synthesized by lymphoid cells. Many other globulins are synthesized by the liver (Jones and Allison, 2007; Russell and Roussel, 2007). The major function of triacylglycerols in animals is a source of fatty acids to be used as metabolic fuel. Mobilization of fatty acids from the fat stores is regulated by hormonal balance which in turn is responsive to nutritional and physiological states (Gurr *et al*., 2002).

Serum glucose in ruminants is lower than nonruminant species because ruminants produced glucose-precursors named volatile fatty acids within the rumen which then are absorbed by the rumen and transported by the blood. Endogenous and exogenous steroids increase gluconeogenesis and serum glucose (Russell and Roussel, 2007). Now-a-days, there is a raising concern of physiological indicators which could help to better understand variations in feed efficiency and to identify most efficient animals. The aim of this project was to evaluate specific hematological and biochemical parameters on high and low-RFI Rambouillet sheep.

MATERIALS AND METHODS

Residual feed intake was calculated on 24 ewes and 16 rams. Animals were fed a high roughage diet with NRC predicted nutrient profile (@ 3.0% BW Dry Matter Intake, (DMI) of DM, 88%; TDN, 68%; ME, 2.48 Mcal day^{-1}; CP, 16%; Ca, 0.7% and P, 0.30%. RFI was calculated for each individual within sex as the difference between actual and expected feed intake. Expected feed intake was modeled:

$$Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MTBW_i^{0.75}$$

Where:

Y_i = Expected daily feed intake of animal i
β_0 = The regression intercept
β_1 = Partial regression coefficient of feed intake on ADG
β_2 = Partial regression coefficient of feed intake on mean test $BW^{0.75}$

Calculations were done using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). After determination of RFI, rams and ewes were classified into highest positive (>0.5 SD above the mean; n = 4 and n = 6, respectively) and lowest negative (<0.5SD below the mean; n = 4 and n = 6, respectively) RFI groups. Samples were taken by trained personnel by external jugular venipuncture at 7 a.m. before feed supplementation to calm animals. Blood samples were taken at day 0, 21 and 42 for determination of hematological parameters and at day 0 and 42 for biochemical parameters evaluation from RFI categorized ewes and rams. Vacuumed tubes with blood anticoagulated with Ethylenediaminetetraacetic Acid (EDTA) were used for hematological analysis. Tubes were filled until end of vacuumed to allow proper proportions of blood-EDTA; these were carefully inverted several times to ensure proper mixing of the blood sample with the EDTA then were allocated over ice to maintain proper temperature until subsequent laboratory analysis (<30 min after blood collection).

Blood tubes without EDTA were transported on ice to the laboratory; these were allowed to clot at room temperature after that clot was removed and serum was separated from cells by centrifugation, then serum samples were allocated on microtubes and frozen at -20 EC for subsequent laboratory analysis.

The packed cell volumes (PCV, %) were determined on each blood sample by centrifugation in microhematocrit capillary tubes and expressed on percentage of RBCs per volume of blood. Hemoglobine was determined with the Spencer hemoglobinometer (Nappert *et al*., 1989). Total red blood cells (10^6 μL) and white blood cells (10^3 μL) were counted directly at the microscope (Benjamin, 1958). Total plasma proteins reported in hematological parameters were determined using the Goldberg refractometer. The mean corpuscular volume:

$$\left(MCV = \frac{(PCV \times 10)}{RBC}\right)$$

Mean corpuscular hemoglobin:

$$\left(MCH = \frac{(Hgb \times 10)}{RBC}\right)$$

And mean corpuscular hemoglobin concentration:

$$\left(MCHC = \left(\frac{Hgb}{PCV}\right) \times 100\right)$$

were calculated. Biochemical parameters were determined in serum with the aid of commercial kits (Diagnostic Chemicals Limited®): total proteins (total protein assay); glucose (glucose-SL assay) and triglyceride (triglyceride-SL assay).

Hematological and biochemical parameters were analyzed as a repeated measure analysis of variance to test effects of RFI (negative or positive), repeated measures of time (day 0, 21 and 42 on hematological parameters and on day 0 and 42 in biochemical parameters) and their interactions using the MIXED procedures of SAS (SAS, 9.1.3). Non significant interaction terms were removed from the model. Least

squares means were computed for the variables and pairwise comparisons were made by using Tukey's W procedure. Pearson correlation coefficients were computed by RFI groups within ewes and rams.

RESULTS

Relationships of positive (less efficient) and negative RFI (more efficient) categorized Rambouillet ewes and rams on hematological (day 9, 21 and 42) (Table 1) and biochemical parameters (day 0 and 42) (Table 2) are presented and discussed. Overall, negative RFI ewes (n = 6) tended to have ($p \leq 0.10$) less RBC (1.98×10^6 μL) and more MCH (3.36 pg) than positive-RFI ewes (n = 6). A day effect ($p<0.05$) was observed on PCV, Hgb and PP. RFI was weakly related with RBC (Pearson correlation coefficient r = 0.35, $p<0.03$), VCM (r = -0.31, $p<0.05$) and MCH (r = -0.43, $p<0.01$). It was also observed a decrement ($p<0.05$) on RBC (2.6×10^6 μL), WBC (2.48×10^3 μL) and MCV (13.13 fL) and weakly effect ($p<0.10$) on MCH (4.62 pg) in negative-RFI rams compared with less efficient rams. A day effect ($p<0.05$) was observed on PCV, Hgb and PP. RFI was related with WBC (r = 0.43, $p<0.02$) and glucose (r = 0.63, $p<0.01$). As well, plasma protein was related to serum protein (r = 0.69, $p<0.001$) in rams. The reference intervals reported for hematological parameters in sheep are: RBC, 9-15×10^6 cells μL^{-1}; PCV, 27-45%; Hgb, 9-15 g dL^{-1}; MCV, 28-40 fL; MCH, 8-12 pg; MCHC, 31-34%; WBC, 4-12×10^3 μL; PP, 6.0-7.5 (g dL^{-1}) (Jones and Allison, 2007).

Less efficient ewes shown ($p<0.01$) more glucose concentrations (mg dL^{-1}) than more efficient also it was observed a tendency ($p<0.10$) decreasing triglycerides (mmol L^{-1}) on negative RFI ewes. Less amount of total proteins were observed at day 42 in comparison with day 0.

Protein from plasma was related to protein in the serum (r = 0.53, $p<0.02$). Moreover, glucose was increased in positive RFI rams in comparison with more efficient ruminants. It was positive related RFI and glucose (r = 0.63; $p<0.01$). The reference intervals reported for biochemical parameters in sheep are: glucose, 2.25-3.29 m mol L^{-1}; total proteins, 6-7.5 g dL^{-1}; triglycerides, 0.1-0.2 m mol L^{-1}.

Table 1: Relationship of residual feed intake on hematological parameters in Rambouillet sheep

	RFI effect				Day effect				
Traits	Negative RFI	Positive RFI	EEM	p value	0	21	42	EEM	p value
Ewes									
PCV (%)	24.23	24.44	0.95	0.830	27.52^a	21.12^b	24.38^c	1.17	<0.001
Hgb (g dL^{-1})	8.36	7.76	0.50	0.240	8.99^A	7.52^B	7.66^C	0.61	0.040
PP (g dL^{-1})	6.31	6.18	0.14	0.390	6.64^a	6.17^b	5.92^b	0.17	<0.001
RBC 10^6 μL	7.68	9.66	1.20	0.100	10.29	8.21	7.52	1.47	0.160
WBC10^3 μL	8.61	7.90	1.04	0.500	8.06	7.45	9.26	1.27	0.340
MCV (fL)	37.91	29.79	5.53	0.150	31.93	30.06	39.57	6.77	0.340
MCH (pg)	12.95^A	9.59^B	1.87	0.080	10.93	10.64	12.23	2.28	0.760
MCHC (%)	35.05	32.47	2.72	0.351	33.72	32.47	33.72	3.34	0.440
Rams									
PCV (%)	25.60	25.00	0.98	0.550	27.90^a	21.70^b	26.30^a	1.20	<0.001
Hgb (g dL^{-1})	9.30	9.30	0.58	0.980	10.50^a	8.25^b	9.15^{ab}	0.71	0.010
PP (g day^{-1})	6.09	6.23	0.13	0.280	6.60^a	5.46^b	6.43^a	0.16	<0.001
RBC 10^6 μL	6.67^a	9.27^b	1.11	0.030	9.35	7.46	7.10	1.36	0.220
WBC10^3 μL	7.77^a	10.25^b	0.76	<0.010	10.15^A	8.91^{AB}	7.98^B	0.93	0.090
MCV (fL)	44.11^a	30.98^b	6.35	<0.050	36.50	32.93	43.20	7.78	0.420
MCH (pg)	16.12^A	11.50^B	2.55	0.080	14.03	12.45	14.96	3.12	0.720
MCHC (%)	36.34	38.04	2.89	0.560	37.86	38.61	35.08	3.53	0.580

^{ABC}Means within rows with unlike superscripts differ $p<0.05$, respectively for RFI and day main effects; ^{abc}Means within rows with unlike superscripts differ $p<0.01$, respectively for RFI and day main effects

Table 2: Relationship of residual feed intake on biochemical parameters in Rambouillet sheep

	RFI effect				Day effect			
Traits	Negative RFI	Positive RFI	EEM	p value	0	42	EEM	p value
Ewes								
Proteins (g dL^{-1})	6.32	6.15	0.170	0.340	6.52^a	5.95^b	0.170	0.003
Glucose (mg dL^{-1})	63.57^a	77.30^b	4.840	0.010	67.99	72.88	4.840	0.330
Triglyceride (mmol L^{-1})	0.16^A	0.19^B	0.017	0.090	0.17	0.19	0.017	0.470
Rams								
Proteins (g dL^{-1})	6.27	6.43	0.230	0.490	6.62c	6.08^d	0.230	0.030
Glucose (mg dL^{-1})	51.44^a	80.23^b	2.370	<0.001	57.13^a	74.54^b	2.370	<0.001
Triglyceride (m mol L^{-1})	0.22	0.18	0.027	0.190	0.16^a	0.25^b	0.027	0.003

^{AB}Means within rows with unlike superscripts differ $p<0.10$, respectively for RFI and day main effects; ^{cd}Means within rows with unlike superscripts differ $p<0.05$, respectively for RFI and day main effects; ^{ab}Means within rows with unlike superscripts differ $p<0.01$, respectively for RFI and day main effects

DISCUSSION

The increased concern of feed efficiency due to a lower availability and high costs of feedstuffs used for animal nutrition has prompted researchers and producers to rethink strategies to improve energy efficiency or to identify most efficient ruminants. Production efficiency has increased in ruminants by the absence of direct selection to improve feed efficiency (Johnson *et al*., 2003). Actual animal improvement has been due to grain-feeding production systems, adoption of nutrition, reproductive and pharmaceutical-based technologies and the application of crossbreeding and selection programs that focused on output traits (Carstens and Kerley, 2009). The lack of an appropriate trait for use in selection programs has also curtailed genetic progress in feed efficiency.

Now-a-days, it is not fully understood differences in animal feed efficiency and there is a lack of biological markers tended to identify most energetically efficient ruminants. Results from the present experiment showed that RBC (10^6 μL^{-1}) increased by 20 and 28% on less efficient ewes and rams, respectively; these values are within reference intervals. The main function of a mammal's spleen is to store red blood cells. It has been reported that stress factors release catecholamine which in turn produces a contraction of the spleen with the subsequent increase in RBC, hematocrite and hemoglobin values (Marco and Lavin, 1999).

Most efficient rams showed a decrement on WBC (10^3 μL) of 24% in comparison with less efficient (positive RFI). A typical stress response in ruminants involves the release of glucocorticoids which stimulate a characteristic change in the pattern of white blood cells. This includes increased neutrophils, a concurrent drop in total white blood cell count as the numbers of lymphocytes, eosinophils and basophils generally also decrease. Richardson *et al.* (2002) also reported changes in hematological profile indicative of cattle that are mildly stressed with the less efficient steers having more neutrophils, fewer lymphocytes and lower white blood cell count, compared with the more efficient steers. Those researchers found significant positive relationships for RFI with hemoglobin and hematocrite which may be a result of contraction of the spleen as occurs following excitation (Gartner *et al*., 1969) suggesting that high RFI (low efficiency) steers are on average more excitable or easily stressed than low-RFI (high efficiency) steers.

Bornez *et al.* (2009) did not found differences in leucocyte count between suckling and weaned lambs, however when animals suffer stress by transportation, leucocyte values tended to increase in comparison with animals at the farm. This response probably associated to higher values of cortisol and adrenaline after transportation, since catecholamines can cause a transient leukocytosis to the circulating pool. Mean Corpuscular Volume (MCV) variations seem to be representative about the possible stress that lambs suffer during different handling procedures (Bornez *et al*., 2009). Further, evidence is the trend for a positive genetic relationship between blood cortisol concentration of ruminants and RFI.

Stress has been reported as inducing energy mobilization in the form of lipid catabolism and protein degradation (Richardson and Herd, 2004). In physiological states demanding the consumption of fuel reserves, the resulting low concentrations of insulin turn off the biosynthetic pathways and release the inhibition of hormone-sensitive lipase within adipocytes, producing triglycerides increment (Gurr *et al*., 2002). However at the present trial, it was not observed an RFI-effect on triglycerides concentrations neither ewes nor rams. However, RFI was weakly related to triglycerides ($r = -0.10$, $p<0.05$).

When plasma is used instead of serum for a chemistry profile, fibrinogen is included in the total protein concentration and is reflected in the calculated globulin fraction (Jones and Allison, 2007; Russell and Roussel, 2007). No effects were observed on total proteins.

Reported reference intervals for serum glucose in ruminants are lower than those reported for nonruminant species. Hyperglycemia occurs with stress including excitement, fear and severe pain; bovine milk fiber, displaced abomasums, etc. Stress-induced hyperglycemia, mediated by epinephrine or endogenous glucocorticoids may be accompanied by transient glucosuria. Endogenous and exogenous steroids increase gluconeogenesis and increase serum glucose. Low levels of glucose concentrations (40-60 mg/100 mL) have been reported on fasting ruminant states which could help to explain the results on negative RFI rams. Hyperglycemia in ruminants has been reported under different stress conditions (Lorenz, 2000), mediated by epinephrine or endogenous glucocorticoids.

At the present trial, glucose increased on 22 and 56% on ewes and rams, respectively. Intake of feed, digestion of feed, metabolism (anabolism and catabolism associated with and including variation in body composition), activity and thermoregulation have been summarized as physiological mechanisms by which variation in RFI may occur (Herd *et al*., 2004).

Also Richardson and Herd (2004) estimated that heat production from metabolic processes, body composition and activity explained 73% of the variation in RFI.

However, it is necessary more research to validate proportional contribution to differences on animal feed efficiency and quantify the stress effect on biological processes.

CONCLUSION

Hematological and biochemical parameters can explain in part biological variations in RFI sheep. Results from this research suggest that more efficient sheep are less susceptible to stress which represent potential physiological mechanisms by which variation in RFI may occur. More research has to be done to quantify the stress effect on feed efficiency. Also alternative strategies to identified superior RFI animals need to be developed.

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REFERENCES

Azab, M.E. and A.H.A. Maksoud, 1999. Changes in some hematological and biochemical parameters during prepartum and postpartum periods in female Baladi goats. Small Rum. Res., 34: 77-85.

Benjamin, M.M., 1958. Outline of Veterinary Clinical Pathology. 3rd Edn., The Iowa State University Press, Ames, Iowa.

Bornez, R., M.B. Linares and H. Vergara, 2009. Haematological, hormonal and biochemical blood parameters in lamb: Effect of age and blood sampling time. Livest. Sci., 121: 200-206.

Cammack, K.M., K.A. Leymaster, T.G. Jenkins and M.K. Nielsen, 2005. Estimates of genetic parameters for feed intake, feeding behavior and daily gain in composite ram lambs. J. Anim. Sci., 83: 777-785.

Carstens, G.E. and M.S. Kerley, 2009. Biological basis for variation in energetic efficiency of beef cattle. Proceedings of the Beef Improvement Federation 41st Annual Research Symposium, April 30-May 3, Sacramento, California, USA., pp: 124-131.

Gartner, R.J.W., L.L. Callow, C.J. Granzien and P.M. Pepper, 1969. Variations in the concentration of blood constituents in relation to the handling of cattle. Res. Vet. Sci., 10: 7-12.

Gurr, M.I., J.L. Harwood and K.N. Frayn, 2002. Lipids as Energy Stores, in Lipid Biochemistry. 5th Edn., Blackwell Science Ltd., Oxford, pp: 93-126.

Herd, R.M. and S.C. Bishop, 2000. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livest. Prod. Sci., 63: 111-119.

Herd, R.M., V.H. Oddy and E.C. Richardson, 2004. Biological basis for variation in residual feed intake in beefcattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric., 44: 423-430.

Johnson, D.E., C.L. Ferrell and T.G. Jenkins, 2003. The history of energetic efficiency research: Where have we been and where are we going. J. Anim. Sci., 81: 27-38.

Jones, M.L. and R.W. Allison, 2007. Evaluation of the ruminant complete blood cell count. Vet. Clin. North Am.: Food Anim. Pract., 23: 377-402.

Koch, R.M., L.A. Swiger, D. Chambers and K.E. Gregory, 1963. Efficiency of feed use in beef cattle. J. Anim. Sci., 22: 486-494.

Lorenz, I., 2000. Retrospective study of serum glucose concentration in cattle with mucosal disease. J. Vet. Med. Ser. A, 47: 489-493.

Marco, I. and S. Lavin, 1999. Effect of the method of capture on the haematology and blood chemistry of red deer. Res. Vet. Sci., 66: 81-84.

Nappert, G., A. Vrins, N. Lariviere and L.N. Ochoa, 1989. Rapid determination of equine hemoglobin concentration with the Spencer hemoglobinometer. Can. Vet. J., 30: 52-53.

Polizopoulou, Z.S., 2010. Haematological tests in sheep health management. Small Ruminant Res., 92: 88-91.

Richardson, E.C. and R.M. Herd, 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agric., 44: 431-440.

Richardson, E.C., R.M. Herd, I.G. Colditz, J.A. Archer and P.F. Arthur, 2002. Blood cell profiles of steer progeny from parents selected for and against residual feed intake. Aust. J. Exp. Agric., 42: 901-908.

Russell, K.E. and A.J. Roussel, 2007. Evaluation of the ruminant serum chemistry profile. Vet. Clin. North Am.: Food Anim. Pract., 23: 403-426.

Shaw, F.D. and R.K. Tume, 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents-a review of recent work. Meat Sci., 32: 311-329.