

Effect of Barley Supplementation on Both Pubertal Age and Metabolic Profile of D'man Lambs Living in a Hostile Environment

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Abstract

Energy and protein adjustment of experimental ration allowed monitoring and recording the reproductive performance (androgen levels and testicular volume) and metabolic profile of D'man breed which is subjected to environmental stress and nutritional control. Lambs were separated in two homogeneous groups and randomly assigned to two diets. In this experiment, each lamb received respectively 250 and 500 g of barley per day in addition to a basic nutrition. Age at puberty is determined by androgens assay and nutritional status is assessed by plasma concentrations of circulating metabolites (glucose, cholesterol, total protein and calcium). Our results indicate that barley supplementation had a good benefit on average daily gain (ADG) on Group B (77.53 versus 51.36 g per day). Puberty is more affected by diet ($P < 0.021$) and lesser extent by age ($P < 0.045$) suggesting that this physiological state would be well correlated with genetic factors that will be relegated to secondary position in opposition to alimentary factor: 66 versus 25 % of lambs of group B are pubescent; they reached 51% versus 68 % of their adult weight, with a rate of plasma androgens corresponding to 2.34 ± 1.20 versus 1.8 ± 0.14 ng/ml. The testicular volume is multiplied by a factor of 11 versus 9. In fact, lambs of Group B reached puberty 90 days rather than those of group A at 5 versus 8 months. The biochemical profiles means are globally identical between the two groups but always in favor of those who received the supplementation. One month after weaning, all biochemical parameters, except proteins of group B, were falling in both groups probably because of the transition to another nutritional state (milk versus concentrate). While, the protein content of group B is high, it remains nevertheless not statistically significant ($P > 0.05$). This increase is probably associated to bypass protein and also to the contribution of the microbial nitrogen at the duodenal flow. The gap between the two groups for reproductive and metabolic characters implies the effectiveness of the diet in terms of energy and protein digestibility. Thus, adjustment of nutrient intakes between the groups A and B supports the hypothesis that food is a major factor unwavering in reproductive events, especially when supplementation coincides with the breeding season.

Keywords: arid area, sheep, supplementation, metabolism, puberty

1. Introduction

The impact of nutrition in the onset of puberty, ovulation and conception is generally well established in mammals. Indeed, the reproductive performances of domestic animals are highly disturbed when energy and protein requirements are either too much or not enough covered, thereby affecting the functioning of this physiological state especially puberty (Lefevre and Bringer, 2005).

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The quantitative nutritional imbalances (excesses or deficiencies) and quality can lead directly to hyper metabolism (obesity) or hypo metabolism (wasting) states that have a negative impact on reproductive activity. In fact, the metabolic status and its relationship to the reproductive axis depend on the pulsatile secretion of LH, itself depending on factors that affect the activity of GnRH neurons. Many hypothalamic peptides are vectors of information transmitted by its internal and external environment as maturity, nutritional status, health status, light, temperature and social structure. Moreover, the effects of nutrition on LH secretion derived from metabolic mediators acting directly and/or indirectly on the hypothalamic-pituitary axis. These mediators are nutrients (glucose, fatty acids and amino acids) or hormones involved in regulating metabolism (insulin, IGF-1, cortisol, thyroid hormones and leptin ...). The integration of social, genetic and nutritional environment is all limiting factors that affect reproductive function. In order to enhance local species, we planned to use a prolific breed for its rational exploitation, genetic potential and adaptive physiology. The desert ruminants are more efficient (Silanikove, 1986) because they would present a greater digestive capacity, an efficient economy of nitrogen and intelligent use of water resources (Silanikove, 2000). The origin of this adaptive phenomenon lies in the recycling of nitrogen. The goal of our work is to explore the reproductive performances (particularly puberty) by adjusting the nutritional intakes in a local ovine species named D'man living in arid area.

2. Materials and Methods

2.1 Study Area

Experiment is conducted at the experimental station (Height: 397mm; Latitude: 30°34'N, Longitude 02°52'E) in southern Algeria. This area is characterized by an arid climate, very hot in summer and cold in winter, temperatures vary between 3.6 and 50 degrees Celsius and humidity close 80% in the wet season and 13% during hot season.

2.2 Food (Barley)

The adaptive ability of barley to arid soils, heat, cold and altitude make it an excellent reference food in the diet of growing-fattening cattle. In addition it contributes to increase the energy values of the diet, its energy and proteins are digested more efficiently than other cereals. For this reason, this crop is used as a base in the diet of our livestock. The physicochemical parameters of barley: water content (ISO.712/1990), ash (ISO.2171/1990), total nitrogen for the calculation of total protein [(NF V18-100 (AFNOR, 1977)], crude fat (ISO.7302/1982) and total carbohydrate (Bertrand method) are determined by the International organization of standardization (ISO) to calculate the energy value of ration.

2.3 Animals and Techniques

All procedures were conducted in accordance with Ministry of Agriculture and Rural Development in accordance with the Directive of the 2010/63/EU animal experiments;

(http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). Twelve lambs were selected among mothers reared and farrowed at the station. During the first months, lambs (Group A: n =6, Group B: n =6) were breastfed and weaned at the age of three months; they were penned into individual boxes and received respectively 250 and 500 g of barley per day equivalent to: 0.25 UF, 22.5 g proteins (PR), 37.5 g crude proteins (CP), 6.6 g fat, 189.82 g total carbohydrate (TC) and 0.50 UF, 45 g proteins (PR), 75 g crude proteins (CP), 13.2 g fat, 379.65 g total carbohydrate (TC) respectively, in addition to a basic diet consisting of alfalfa hay and rubbish scrap dates. Morphological data of body weight and testicular volume are collected monthly and stored since the beginning until the end of the experiment (1 to 12 years). Lambs are isolated and the blood was collected monthly from the jugular vein, in vacutainer heparinized tubes and centrifuged at 3000 g. The samples were stored at - 20 ° C until assay. Plasma androgen concentrations are determined by radioimmunoassay, using the technique described by Berson and Yalow (1959) and validated in our laboratory: 400 µl of plasma are extracted with 2.5 ml of a cold mixture of cyclohexane-ethyl acetate (V/V). Losses estimated by calculating the percentage recovery are at 98.18%. The sensitivity limit is 0.94 pg/tube and the coefficients of variations of intra- and inter-assay were respectively 6.96% and 12.02%. We defined the onset of puberty when plasma androgen exceeded 1 ng/ml. Glucose, proteins, cholesterol and calcium levels are estimated by a general protocol for colorimetric assay (RANDOX Kits, Crumlin, Northern Ireland, and United Kingdom). The color intensity is proportional to the concentration of analytes assayed.

2.4 Statistical Analysis

Numerical results are shown as arithmetic means with their standard errors ($X \pm \text{ESM}$). The effects of age (months), diet (group) and their interaction (Age * Diet) were studied using analysis of variance (ANOVA) on XLSTAT 2009 Software. Means were compared using the Fisher (LSD) test. All results were considered significant at $P \leq 0.05$.

3. Results

3.1 Chemical Composition of Barley (*Hordeum Vulgare*)

Unlike to corn and soy, barley is an excellent cereal, because it allows a gradual and functional adaptation of the intestinal mucosa; increases the activity of some enzymes responsible of the absorptive capacity of glucose and prevents the acceleration of transit (diarrhea and sometimes death) often observed when switching from liquid to solid food. It has an intermediate energy value between corn (365 calories) and wheat (338 calories); this choice was dictated by both digestive and caloric properties (Table1).

Table 1: Chemical Composition of Barley (*Hordeum Vulgare*)

Energetic value per 100 g of barley analyzed =352 calories	
Dry matter (DM)%	87,68
Humidity (H)%	12,32
© ash%	2,26
Total Carbohydrates (TC)%	72,93
Total lipid (fat)%	2,64
Total protein (PR)%	9,01
Crude protein (CP)%	15

3.2 Curve Growth

3.2.1 Body Weight (Kg)

After the introduction of new diet, the gain weight of group B was 72 kg whereas those of the group A was only 31 g (data is not available). The growth and development of D'man sheep reveal morphological changes at the age of seven months in both groups which were confirmed by their significant values ($P < 0, 05$). The evaluation and monitoring of the growth curve showed that they have reached 51-68 % (Table 2) of their adult weight at 5 (B) and 8 (A) months (16.67 ± 1.28 versus 16.70 ± 1.28 kg), age corresponding probably to puberty (FIG 1).

Table 2: Means Values in Biometric and Testicular Parameters [(Body Weight (kg), Testicular Volume (ml) and Plasmatic Androgens (ng.ml⁻¹)] ; Group C (lambs before Sharing and Weaning)

Variable	Biometric and testicular parameters						
	Age month	Body weight (kg)		Testicular volume (ml)		Plasmatic androgens (ng.ml)	
		Group C		Group C		Group C	
Initial age	M1	6.3 ± 0.83		6.04 ± 1.65		0.092 ± 0.004	
Weaning age	M3	11.48 ± 1.42		22.59 ± 5.95		0.52 ± 0.03	
Pubertal age (Group B)	M5	-	16,67 ± 1,28 *	-	69,15 ± 5,60*	-	2,34 ± 0,20 *
Pubertal age (Group A)	M8	16,70 ± 1,28	-	55,57 ± 11,39	-	1,18 ± 0,14 *	-
The end of experiment	M12	25,00 ± 1,08**	33,00 ± 0,57 ***	133,74 ± 20,96 *	157,21 ± 16,95 **	0,41 ± 0,09	1,27 ± 1,02

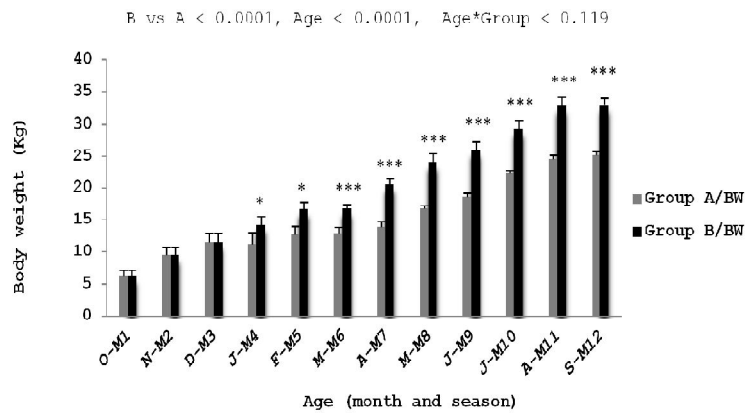


Figure 1. Evolution of the growth curve of D'man lambs supplemented (BW: body weight, Group A: supplemented with 250g/day, Group B: supplemented with 500g/day, M: months; Group: diet, M*Group: interaction): *P < 0.05; **P < 0.01; ***P < 0.001

3.2.2 Average Daily Gain (ADG) Expressed in Grams per Day (g/day)

During the lactation phase (M1-M3), the twelve lambs showed a growth rate approximately of 94.18 g/d, against only 51.36 versus 77.53 g/d, respectively for lambs A and B during the post-weaning period (M3-M12), in favor of group B (Table 3). The gain difference observed between lactation and growth phases in two groups is of 42.82 (B) versus 16.5(A) g/d.

Table 3: Means Values of Average Daily Gain (g/d) in A, B and C groups

Average daily gain (g/d)			
Lactation's stage	M1-M3	94,18 g/d (C)	
Weight gain after one month withdrawal	M3-M4	7,86 g/d (A)	97,14 g/d (B)
Post-weaning stage	M3-M12	51,36 g/d (A)	77,53 g/d (B)
Overall phase of study	M1-M12	59,22 g/d (A)	80,63 g/d (B)

3.3 Testicular Parameters

3.3.1 Testicular Volume

Testicular volume increased immediately after the attribution of the concentrate to achieve rates of 24.41±11.46 (A) versus 37.86±11.50 ml (B). At five months (the presumed age of puberty), testicular volume of lambs in group B increased to 69.15 ±5.60 ml against 55.57±11.39 ml for group A (Table 2). The statistical study shows that this parameter is strongly influenced by the age factor (P < 0.0001) and least by the food factor (P < 0.001). In addition, we observed two inflections points at 5 and 6 months for Group B and at 8 and 9 months for Group A probably suggesting a proliferation of testicular parenchyma (FIG 2).

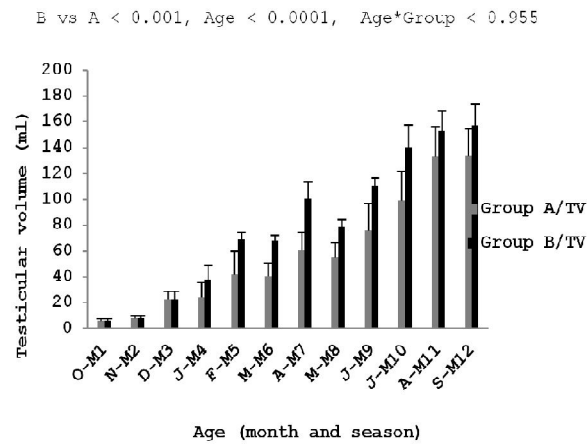


Figure 2. Evolution of Testicular Volume of D'man lambs supplemented (TV: testicular volume, Group A: supplemented with 250 g/day, Group B: supplemented with 500g/day, M: months; Group: diet, M*Group: interaction); *P<0.05; **P<0.01; ***P<0.001

3.3.2 Plasmatic Androgens

Mean plasma concentrations of androgens vary between 0.58 ± 0.15 and 1.07 ± 0.26 ng/ml respectively for group A and B. The first significant difference observed in this study allowed locating the age at puberty. The low rate observed at the beginning of experiment increases to achieve a first level corresponding probably to weaning (0.516 ng/ml). Two significant values ($P < 0.02$ versus $P < 0.046$) appear at two distinct periods 5 and 8 months (2.34 ± 0.20 versus 1.18 ± 0.14 ng/ml); this difference seems to be largely due to the composition of the diet and introduce the notion of puberty (Table 2). At the age of 11 months, the values reach two peaks (1.77 versus 3.00 ng/ml in both groups) probably indicating the full breeding season (FIG3).

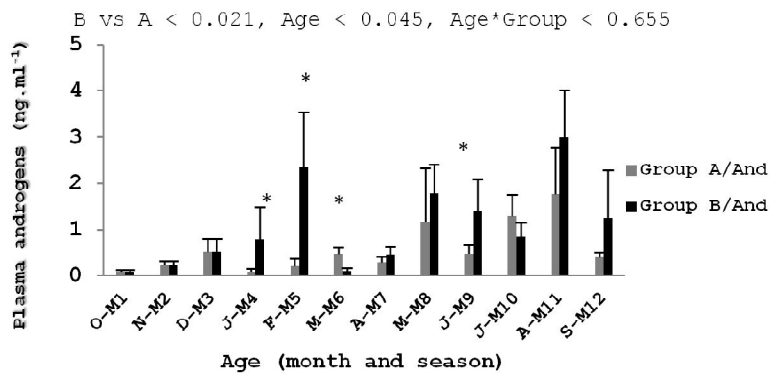


Figure 3. Evolution of plasmatic androgens of D'man lambs supplemented: (And: androgens, Group A: supplemented with 250g/day, Group B : supplemented with 500g/day, M: months; Group: diet, M*Group: interaction) ; * P<0.05; **P<0.01; ***P<0.001

3.4 Biochemical Metabolites

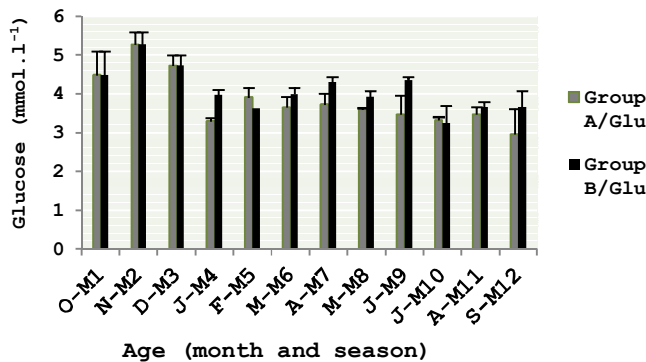
In this study, we investigated the expression of biochemical parameters measured firstly between lactation and post-weaning phases and secondly in both groups after the award of the concentrate. Except the cholesterol [1.51 ± 0.17 (B) versus 1.56 ± 0.19 (A) mmol/l] which show values more or less steady in the two groups (M1-M12), the other parameters such as glucose, proteins and calcium are significantly higher in the group B (4.10 ± 0.24 versus 3.83 ± 0.30 mmol/l, 606.06 ± 51.41 versus 590.74 ± 40.30 g. dL⁻¹, 2.13 ± 0.29 versus 1.93 ± 0.30 mmol/l).

In fact, the average circulating metabolites namely glucose, cholesterol, proteins and calcium between M1 – M3 ($4,83 \pm 0,40 \text{ mmol.l}^{-1}$, $2,66 \pm 0,28 \text{ mmol.l}^{-1}$, $622,48 \pm 69,34 \text{ g.l}^{-1}$, $2,23 \pm 0,35 \text{ mmol.l}^{-1}$) are generally higher than those observed between M4- M12 for both groups A and B ($3,49 \pm 0,26$ versus $3,85 \pm 0,19 \text{ mmol/l}$, $1,19 \pm 0,16$ versus $1,12 \pm 0,14 \text{ mmol/l}$, $580,16 \pm 30,62$ versus $600,59 \pm 45,43 \text{ g.dL}^{-1}$, $1,83 \pm 0,29$ versus $2,09 \pm 0,26 \text{ mmol.l}^{-1}$) (Table 4). This implies that the energy value of the concentrate cannot compensate the nutritional value of breast milk. In addition, all biochemical parameters show a fall, one month after weaning, with the exception of total proteins of group B that remain more or less stable probably due to the turnover of rumen bacteria. Furthermore, similarities in changes observed between the two groups suggest that they follow probably seasonal variations. We also note that all biochemical parameters analysed are influenced by age with the exception of glucose which would be more sensitive to the energy content of the diet (FIG 4).

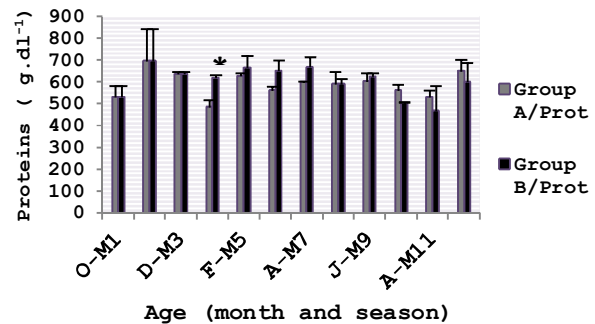
Table 4: Means Values in Biochemical Parameters

Parameters	Lactation stage (M1-M3)	Post-weaning stage (M4-M12)		Overall phase of study (M1-M12)	
	Group C	Group A	Group B	Group A	Group B
Glucose (mmol.l ⁻¹)	4,83 ± 0,40	3,49 ± 0,26	3,85 ± 0,19	3,83 ± 0,30	4,10 ± 0,24
Cholesterol (mmol.l ⁻¹)	2,66 ± 0,28	1,19 ± 0,16	1,12 ± 0,14	1,56 ± 0,19	1,51 ± 0,17
Total protein (g.dl ⁻¹)	622,48 ± 69,34	580,16 ± 30,62	600,59 ± 45,43	590,74 ± 40,30	606,06 ± 51,41
Calcium (mmol.l ⁻¹)	2,23 ± 0,35	1,83 ± 0,23	2,09 ± 0,26	1,93 ± 0,30	2,13 ± 0,29

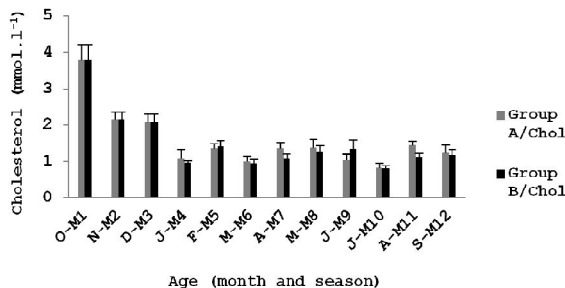
B vs A < 0.003, Age < 0.008, Age*Group < 0.858



B vs A < 0.353, Age < 0.020, Age*Group < 0.335



B vs A < 0.901, Age < 0.0001, Age*Group < 0.610



B vs A < 0.115, Age < 0.003, Age*Group < 0.050

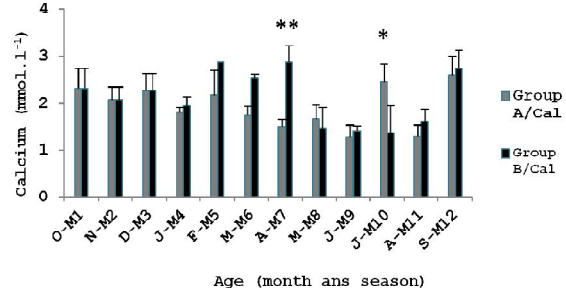


Figure 4. Changes in circulating Glucose (Glu), Cholesterol (Chol), Proteins (Prot) and Calcium (Cal) according to age, diet and season (Group A: supplemented with 250g/day, Group B: supplemented with 500g/day, M: months; Group: diet, M*Group: interaction); *p<0.05; **p<0.01; ***p< 0.001

4. Discussion

Our study allowed assessing the growth and sexual activity of D'man lambs through the adjustment of rations. In fact, the growth performances are closely related to fertility, size and litter weight at birth and size and litter weight at weaning. The growth index lambs D'man (94.18 g/d) from the beginning of the experiment (1 month) until weaning age is slightly lower but remains however much higher than the post-weaning period (Table 2). Contrary to our expectations, the introduction of concentrate, generally marked by the acquisition of weight, has shown the weakness in group A, probably due to the body condition score during the introduction of lambs in experimental herd; this state has already been observed in other breeds of sheep (Gbangboche et al., 2005). This phenomenon called "abstinence syndrome" usually occurs when the lambs are frankly separated from their mother. We noticed that the growth rate of lambs is faster and weight is more important in group B than in group A. This fact can probably be explained by the influence of other factors (maternal age at first lambing, sex, mode and season of birth) (Susic et al. 2005. Rekik et al., 2008). The highly predictive biological and nutritional indicators used in our study helped to analysis quantitatively and qualitatively biochemical curves through the nutritional impact of food introduced into the diet and its projections on the post-weaning phase. Indeed, we note that the average daily consumption of barley (250 vs 500 g/d) distributed to the two groups A and B failed to compensate average daily gain (ADG) [(51.36 (A) versus 77.53 g/day (B))] of lambs during the lactation phase (94.18g/day) (Table 2). This observation shows that the energy value of barley cannot compensate the nutritional value of breast milk.

This discrepancy observed between the two phases can be explained by the adjustment of energy metabolism according to the new dietary data. Requested by Ruminant bacteria, the carbohydrates from plants component, are transformed to essential of their energy needs, under form volatile fatty acids VFA (propionate, acetate and butyrate) precursor of glucose (Bell and Bauman, 1997). The content of the ration of parietal compounds can explain the low circulating levels of glucose observed in both groups in our experiment. Nevertheless, it is important to remember that the need for glucose in growing lambs is determined by their growth rate, which is set by the metabolizable energy. The contribution of proteins bypass degraded and synthesized *de novo* by rumen bacteria did not have the same impact on the growth of lambs than on the stage of lactation. The low caloric diet which characterized group A, shows a reduction in circulating levels of glucose in agreement with its direct precursor the propionate, a situation already discussed by (Reynolds et al., 2003). In addition, the negative energy balance observed in the control group (A) was probably caused by early withdrawal of lambs. Lambs have mobilized their body reserves acquired during lactation, but unfortunately did failed to compensate the weight loss; this phenomenon, has not been observed in lambs in group B. Cholesterol level shows, as glucose, a distinct variation between the breastfeeding phase and the post-weaning, this difference is explained by the chemical composition, texture and nutritional value of the food. We could explain this case by the maximum cholesterol level observed during the lactation phase which corresponds to the maximum body fat. A positive correlation is observed between cholesterol, quality of food and age confirmed in both groups of lambs at least for age factor ($R_a = -0.6549$ $P = 0.021$; $R_b = -0.6973$ $P = 0.012$) and in deer Croatia (Poljicak-Milas et al., 2004). Small changes in cholesterol are not entirely clear, but can probably be attributed to these metabolic interrelations with glucose levels.

To understand and explain the changes observed in the energy balance, it seemed important to analyze the different phases studied in relation to nutritional status. For example, in the case of an undernourishment, the non esterified fatty acid (NEFA) are released into the circulation in response to a lack of energy; these are extracted from lipid reserves, a phenomenon that has been observed in lot of species, in opposition to the growth-fattening phase, an increase in cholesterol esters is observed in other mammals (Heins et al., 1986). The results obtained in the D'man show that lipemia is low at birth, then increases sharply during the first days of life. This ratio is reversed after the introduction of the solid food during the post-weaning phase and remains more and less stable as observed in the pre-ruminant calf and lamb. (Noble et al., 1971). In general, lipemia is influenced by the concentration and the composition of fatty acid of milk. During lactation, the main changes are an increase in blood phospholipids and a decrease in cholesterol esters and a sharp fall in free fatty acids (FFA) after weaning; this explained the progressive decrease in milk supply for the benefit of a solid food. In D'man lambs, proteins are not associated with large fluctuations; their blood levels vary slightly but still have a high diagnostic value in the assessment of nutritional status. It would be interesting at this stage to measure urea, considered as an excellent predictor because of the importance of protein intake and efficiency in small ruminants.

This diagnostic tool allows a retrospective analysis of biological responses to protein and energy supplementation. In ruminants living in arid areas, as is the case for D'man, when food is low in protein, the amount of urea excreted is low; the animal has a recycling capacity of this parameter which can compensate dietary protein deficiencies and to maintain an adequate protein synthesis in the rumen. The continuous supply of the small intestine by protein comes mainly from the rumen microbial synthesis. A few weeks after weaning, the contribution of microbial nitrogen in duodenal flow reached levels seen in adults (Leibholz, 1975; Guigley *et al.*, 1985) and therefore the gastrointestinal tract synthesizes about 30% total protein in lambs weaned. The works of Lallès *et al.* (1990) confirm and show a transient depression and heterogeneous distribution of amino acids in calves during the gradual substitution of milk with solid foods (food protein sources); this, combined with low consumption of dry matter before and just after weaning, may explain circulating protein levels and the observed difference between the two phases of our study. The optimum levels of proteins in rations for growing and fattening is not well documented, but it recommends amounts ranging from 14 to 16% crude protein for weight between 15 -30 Kg. To meet requirements and estimate the needs of lambs, a physico-chemical analysis of barley was performed. Intake of crude protein at lambs during the experiment at all periods was 15% (CP) ie 9% protein. Thereby, lambs fed with 250 g/d of barley (22.5 g protein) consume significantly smaller quantities (g/kg BW^{0.75}) than those fed with 500 g per day of barley (45 g of proteins). This condition seems to affect the Average Daily Gain (ADG) (51.36 vs. 77.53 g/d) to Group B lambs that would seem better manage feed conversion; the modest gain observed in lambs of group A probably reflects an increase in the duration of the period of growth due to nitrogen deficiency (Black *et al.*, 1973).

The involvement of calcium as a diagnostic value in determining the nutritional status of the animals is not excluded, given its close relationship in the various functions of the metabolism (tissue growth, homeostasis ... etc.). Indeed, in domestic ruminants, the need for calcium in young growth is closely linked to the production of breast milk, which increases with the stages of lactation. The first months of life, lambs feed exclusively on milk, the only source of vitamin D; this hormone ensures the intestinal absorption of both calcium and phosphorus. The high rate of this mineral in lactation phase would suggest that there is a specific neonatal maturity species cited by Colston *et al.* (1988) and demonstrated through the administration of 25 (OH) D tritiated which is immediately converted into its active metabolite. In addition to vitamin D, significant amounts of thyroxine (T4) were also identified in colostrum and milk; its presence would contribute to the physiological processes of bone formation and assimilation of calcium and phosphorus in young growing. In our work, after weaning, the relatively low level of calcium observed in the two groups can be explained by the amount of dry matter intake; in fact, the use of the high value silage associated with a lactic acid and pH acid probably facilitates calcium absorption. To overcome environmental stress, thyroid hormones are released continuously to adjust the basal metabolism whose primary objective is to maintain the body temperature via high levels of circulating metabolites. Sexual activity of D'man lambs resulted in a biphasic growth curve, the first is the extension of the linear relationship between testicular weight and the second involving body weight. This curve is characterized by a change in slope at 120 days (group B) with a rapid and progressive increase between 90 and 180 days, suggesting that the lambs in group B began their sexual development. The combined increase in volume and testicular androgens after 90 days probably reflect an increase in the number of Leydig cells (Amman, 1983).

Their differentiation and their maturation begin around the age of 60-90 days (2-3 months) and continues beyond 150 days (5 months). This explains the high rate of testicular androgens between 3-6 months for group B and between 7-8 months for Group A. These results are similar to those reported by (Celi *et al.*, 2006). Indeed, the expression of hypo and hypercaloric diet on the concentration of plasma androgens showed a significant close relationship between weight gain and level of body reserves. The relevance of this phenomenon is explained by the increase (50%) of the frequency of pulses of GnRH and LH in response to protein-energy supply; the reaction stabilizes 5-7 days after the start of the food treatment (Martin *et al.*, 1994). In addition, the stimulatory effect of supplementation on the gonadotropic axis decreases and disappears beyond the 3-4 weeks and this in spite of a continuous supplement, presumably with the onset of weight gain and fat deposition. The increase and the sensitivity of the testicular receptor (LH-R) to gonadotropin during sexual development observed in many species such as dog Inaba *et al.* (1994) between 90 and 150 days Yarney and Sanford (1989) might explain the phenomenon of saturation. In fact, variations in testicular size induced by nutrition are accompanied by changes to the production of sperm by testis. In fact, the testicular activities imply a close synergy between photoperiod, climate and nutrition.

The difference observed between the two nutritional states confirms the sensitivity of somatic and germinal populations cells in their immediate environment. When the body receives insufficient nutritional supplements, this formulation affects the rate of cell division, causing both a reduction in body weight and testicular weight. In addition, tissue sensitivity to food has been widely studied in Merino rams; the testicular response to nutrition also occurs on the diameter of the seminiferous tubules in adults Hotzel *et al.* (1998) which are strongly correlated to the number of germ cells and to the length of Sertoli cells. Our study has showed that biometric, biochemical and androgenic profiles in favor of the lambs of group B, involved directly the effectiveness of the diet, which has provided the maximum energy and protein digestible in the small intestine (PDI). In addition, Nutritional factors also play an important role in the secretion of some hormones and growth factors in the establishment of the physiological process. According to Demigné *et al.* (1988), a strong synergy would exist between the availability of propionate, glucose, insulin and growth of ruminants. The insulin and glucagon are the main vectors of the action of absorbed nutrients on the metabolism. Thus, reducing protein in the diet would result in lower insulin levels as demonstrated by Grizard and Szczygiel (1983a) in lambs of 4 months. The enrichment of the concentrate ration (between 20 and 80%) increases the glucose production by the digestion of dietary carbohydrates in the small intestine. Events involved in growth are closely linked to genetic program, environment and substrates in the diet. In ruminants, insulin is one of the most important hormones during the growth and undergoes many changes. It was showed that his postprandial rise was much greater in the pre-ruminant than in the ruminant; its circulating level can be explained by the reduction of its metabolic clearance rate observed in lambs weighing between 20 and 33 kg. (Grizard Szczygiel, 1983b; Weekes, 1986) reported that insulin receptors in the liver and red blood cells decreased suggesting a reduction of tissue response to the hormone during development. The protein turnover during growth depends on the stage of development (fetal, pre-ruminant growth ruminants, adults) and the support growth requires both a nutrient availability in the quality and quantity and a high concentration of insulin.

5. Conclusion

Our results clearly show that the physiological and metabolic responses to supplementation (500 g/head/day) for the acquisition of puberty have implicated protein composition and energy efficiency of the experimental diets in terms of quality and quantity because the anthropometric parameters; body weight and average daily gain are widely in favor of the supplemented group (B). Thus, testicular parameters (testicular volume and plasma androgens) show a significant increase after the award of concentrated reflecting the direct or indirect involvement of dietary factors on the quantitative and qualitative development of testicular content. This aspect is reflected in the early onset of puberty in the experienced versus control group. Glucose, cholesterol, calcium and protein contents in the concentrate were diverted and processed intelligently by rumen flora to metabolites in order to manage the growth of lambs in particular those in group B. More, we realized that the constitutional value of breast milk is widely rich in essential fatty acids and amino acids that give milk a high energy which could not be offset by the content of the ration after the introduction of solid food.

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