



## Effect of bovine colostrum administration on plasma protein profile, growth, and survival in Red kid



Harouna Abdou<sup>a,1</sup>, Hamani Marichatou<sup>b,2</sup>, Jean-François Beckers<sup>c,3</sup>, Isabelle Dufrasne<sup>a,1</sup>, Jean-Luc Hornick<sup>a,\*</sup>

<sup>a</sup> Department of Animal Production, Faculty of Veterinary, University of Liege, 20 Boulevard de Colonster, B43, 4000 Liege, Belgium

<sup>b</sup> Department of Animal Production, Faculty of Agriculture, University Abdou Moumouni, PO Box 10960, Niamey, Niger

<sup>c</sup> Department of Science Functional, Faculty of Veterinary, University of Liege, 20 Boulevard de Colonster, Building B41, 4000 Liege, Belgium

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### ABSTRACT

This study evaluated the effect of colostrum from Azawak cows on plasma protein profile, growth, and survival in Red kids from Niger. Forty (40) newborn kids were allocated to one of two treatment-groups: control (free access to water and the mother) and colostrum (free access to water and the mother, but with additional 50 mL of colostrum/animal/day of birth and 25 mL/animal/day from the 2nd to 15th day of age). Blood samples were collected into EDTA vacutainer tubes by jugular puncture at 10 and 30 days of age. Total protein was quantified by the Biuret method. The agarose gel electrophoresis was used to determine the serum levels of albumin,  $\alpha$ -globulin,  $\beta_1$ -globulin,  $\beta_2$ -globulin,  $\gamma$ -globulin and the albumin/globulin ratio. The animals from the colostrum group showed higher body weight and average daily gain when compared to the control group ( $P < 0.001$ ). The average concentration in protein at the both sampling times reached higher values in the colostrum than in the control group. At D10, the colostrum group tended ( $P < 0.07$ ) to show higher levels of  $\alpha$ -globulin and had higher values ( $P < 0.05$ ) for  $\beta_1$ -globulin. At D30, total protein and  $\beta_2$ -globulins were higher in colostrum group. Colostrum from Azawak cows seems to have positive effects on some plasma proteins levels and on growth rate in Red kids.

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### 1. Introduction

In ruminants, owing to the epitheliochorial nature of the placenta (Silim et al., 1990), the transmission of antibodies

to fetus fails during the pregnancy (Rawal et al., 2008). At birth, newborn is thus gamma-globulin deprived (Castro et al., 2005) while shifting from a healthy to a hostile environment (Alloncle, 1980; Rawal et al., 2008). The survival of young animals is of great concern for producers. As a rule, the mortality rate is higher from zero to three months of age in goat (63.4% in 2010 at the Secondary Goat Farm Center of Maradi) and other species (N'Diaye-Wereme et al., 1998). These losses greatly affect the profitability of the farm.

Colostrum administration is vital for newborn (Marion et al., 2002; Stelwagen et al., 2009), especially for twins (Hamadeh et al., 2013; Nowak and Poindron, 2006). Several authors (Tuscherer et al., 2000; Edwards, 2002; Le Dividich et al., 2004) showed that defect in colostrum intake within 24–48 h (h) after parturition is related to newborn casualties. In Niger, where rearing conditions are precarious,

\* Corresponding author. Present address: Laboratory of Nutrition, Department of Animal Production, Faculty of Veterinary, University of Liege, 20 Boulevard de Colonster, B43, 4000 Liege, Belgium.  
Tel.: +32 043664131; fax: +32 043664122.

E-mail addresses: hanafiou82@yahoo.fr (H. Abdou), marimani\_m@yahoo.fr, maricha@refer.ne (H. Marichatou), jfbeckers@ulg.ac.be (J.-F. Beckers), Isabelle.Dufrasne@ulg.ac.be (I. Dufrasne), jlhornick@ulg.ac.be (J.-L. Hornick).

<sup>1</sup> Tel.: +32 043664131; fax: +32 043664122.

<sup>2</sup> Tel.: +227 93 91 65 31; fax: +227 20 316612.

<sup>3</sup> Tel.: +32 043664161; fax: +32 043664165.

dairy females do not produce enough colostrum and milk that could cover the needs of the newborns, enhancing the risk for a young animals to die. To our knowledge, no work focused on the production of colostrum in the Red goat. But Oumara (1986) indicated that its average daily milk production reach only 0.75 kg/d. It is possible to assist kids with bovine colostrum (N'Diaye-Wereme et al., 1998; King et al., 2008). Administration of heterologous colostrum also has been yet experienced in pig with good results (Huguet et al., 2007). Indeed, the bovine colostrum contains growth promoters and immunological compounds potentially useful for other species such as piglets, kids and lambs (Godden et al., 2009). Bovine colostrum supplementation in goat is fewly documented in the literature, especially at plasma level. The aim of this study was thus to evaluate the impact of an Azawak colostrum load in Red kids on their plasma protein profile, growth rate, and survival.

## 2. Materials and methods

### 2.1. Experimental site

The study was conducted at the Secondary Goat Farm Center of Maradi (SCGFM) in Niger, from September 2011 to October 2011. Average ambient daytime temperature is 35 °C and annual rainfall is between 600 and 700 mm. The town of Maradi is located about 600 km south-east of Niamey between 13° and 15°26' North and 6°16' and 8°33' East. SCGFM is located 3.5 km East of Maradi and covers an area of 1850 ha. Semi-intensive rangelands used are composed, according to Naba (2001), of a herbaceous cover (*Andropogon gayanus*, *Cenchrus bifloris*, *Eragrostis tremula*, *Commelina forskaeae*, *Corchorus tridens*, *Jacquenontia tamnifoni*) and ligneous species (*Sclerocarya birrea*, *Polystigma reticulatum*, *Acacia albida*, *Acacia nilotica*, *Guiera senegalensis*, *Annona senegalensis*, *Balanites aegyptiaca*, *Boscia senegalensis*, *Combretum glutinosum*, *Combretum micraham*, *Prosopis africana*, *Tamarindus indica*).

### 2.2. Colostrum collection and analysis

The colostrum samples were obtained from two multiparous Azawak zebu cows (second lactation number), within one hour of calving. The cows were from the Sahelian Experimental Station of Toukounous (SEST) located 200 km north Niamey (14°31' North and altitude 3°18' East longitude). They were vaccinated against Contagious Bovine Pleuropneumonia (CBPP). The animals were on rangeland dominated according to Chaibou (2005) by grasses (*Aristida mutabilis*, *A. mutabilis*, *Cenchrus biflorus*, *Eragrostis tremula*, *Schoenfeldia gracilis*, *Panicum laetum*) and wood (*Maerua crassifolia*, *Salvadora persica*). The pregnant and dairy females were supplemented during the dry season with cotton seed (2 kg). The colostrum was analyzed for chemical composition and immunoglobulins content (Table 1). Colostrum immunoglobulins (Ig) and lactoferrin (Lf) were measured at the Center of Rural Economy of Marloie (Belgium) by ELISA, following the manufacturer's recommendations (Bethyl® quantitative sandwich ELISA, USA). Dry matter (DM), crude Ash, nitrogen-free extract (NFE), ether extract (EE) and total nitrogenous

**Table 1**  
Components mean concentrations (g/L).

Composition (g/L)	Means ± SD (n = 7)
IgG	22.5 ± 9.7
IgA	3.3 ± 2.1
IgM	1.7 ± 0.8
Lactoferrin	0.2 ± 0.1
DM	149.8 ± 15.3
CP	67.6 ± 9.2
EE	28.9 ± 0.2
NFE	43.4 ± 3.2
Ash	9.9 ± 0.7
Ca	1.3 ± 0.2
P	1.3 ± 0.2
K	1.4 ± 0.2
Na	0.7 ± 0.2
Mg	0.2 ± 0.0

matter (TNM) were measured according to the methods of the Association of Official Analytical Chemists (AOAC, 2006). Calcium (Ca) and magnesium (Mg) were determined by atomic absorption, potassium (K) and sodium (Na) by flame emission and phosphorus (P) spectrophotometrically.

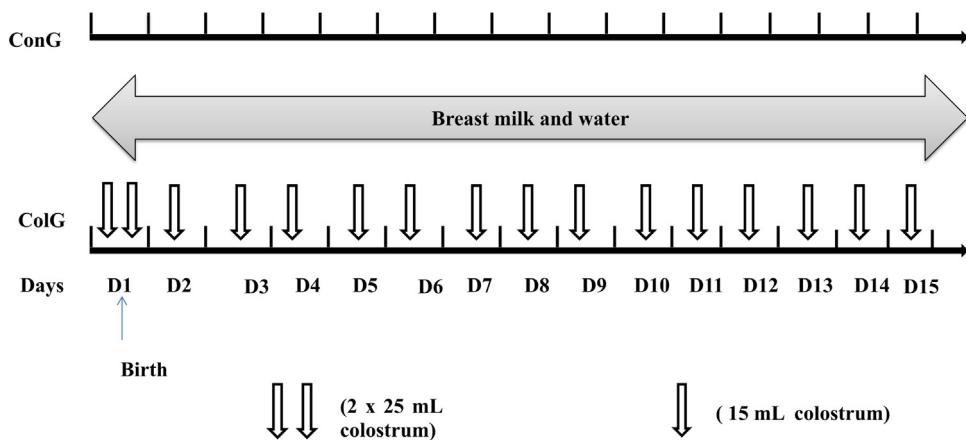
### 2.3. Animals and experimentation

The experimental protocol was approved by the laboratory of Animal production/Faculty of Agriculture/University of Abdou Moumouni (Niamey, Niger) in collaboration with the directorate of Niger's Veterinary Services. The experimental kids were obtained from 9 singletons and 11 twins kiddings. Singletons were alternatively allocated to either the control or the experimental group and twins were randomly allocated to either group, but sex ratio was preserved insofar as possible. The control group (ConG) had free access to water and the mother and the colostrum group (ColG) were offered in nursing bottle additional 50 mL of Azawak colostrum/animal the day of birth (D1) within 12 h of kidding and 15 mL colostrum/animal/d from the D2 to D15. During the day, kids were maintained on semi-intensive rangeland. They were kept at night in permanent shed. Animals grazed under the guidance of shepherds and at night they received biweekly a supplement of concentrates (wheat bran, cottonseed meal). In addition, they had free access to licks in racks. Water was given ad libitum. Each animal was identified by an alpha-numeric number indicating the affiliation to the park, the row of birth, and the sex. Fig. 1 presents the experimental design of the study.

### 2.4. Measurements and samples

The animals were weighted weekly using a balance with maximum load 150 ± 0.1 kg and death occurrences were registered daily.

Blood samplings were performed at D10, the moment the jugular veins was easy to access and at D30. Samples (10 mL/animal) were performed by jugular puncture in EDTA vacutainer tubes (Terumo Corporation, Tokyo, Japan). Samples were transferred 40 min after collection to the laboratory and centrifuged at 1000 rpm for 25 min. Plasmas



**Fig. 1.** Experimental design.

obtained were stored in Eppendorf at  $-20^{\circ}\text{C}$  until analysis at the Biochemistry Unit of the Faculty of Veterinary Medicine, University of Liege, Belgium. Total plasma protein was obtained by the Biuret method. Protein fractions (albumin,  $\alpha$ -globulin,  $\beta_1$  and  $\beta_2$  globulins,  $\gamma$ -globulins) were separated by electrophoresis. The albumin/globulin ratio was calculated.

Electrophoresis was performed on agarose gel following the manufacturer's recommendations (Hydragel protein K20, SEBIA, Lisse, France). Samples were thawed at  $20^{\circ}\text{C}$  for 30 min. The procedure was performed by zone electrophoresis in which the migration is carried on horizontal support made of a porous medium, a strip of blotting paper and the agarose gel. Ten microliters of plasma were put for 4 min in numbered wells. The films were transferred to an electrophoresis tank (K20 SEBIA, Reference No. 1400) during 20 min at 90 V. After migration and revelation films were placed in a colored bath (Coomassie Blue) approximately 5 min and then distained in acetic acid and dried at  $80^{\circ}\text{C}$  in an incubator-dryer (IS 80 SEBIA, Reference No. 1420) while 15 min. The use of phoresy software (device used to view the various profiles) allowed the scanning of films to establish the electrophoresis curves showing the concentration rate of protein fractions. The separation points between profiles of plasma fractions were scored manually on phoresy software. All samples were analyzed by the same people. The absolute concentrations (g/L) of fractions were calculated by multiplying the percentage of each fraction by the total protein concentration determined before.

## 2.5. Statistical analysis

Body weight (BW), average daily gain (ADG) and plasma proteins data were analyzed according to a mixed model (SAS, 1999) including the effects of treatment, time, sex and their interactions. This model included a type 1 auto regression covariance structure for repeated data measured on the same animal. Means were compared using a Student's *t* test with Tukey adjustment. Changes with time in concentration levels were compared according to contrast analysis. The selected threshold of significance was

fixed at 5%. As for survival, data were analyzed according the chi square test.

## 3. Results

### 3.1. Plasma protein

**Table 2** presents least squares means  $\pm$  standard error for total plasma parameters concentrations and ratio in ColG and ConG. The group effect tended to be significant for total protein levels (58.5 vs 55.6 g/L in ColG and ConG respectively,  $P < 0.06$ ) but no time nor interaction effect was observed. At D10, ColG tended to have higher values than ConG (+2.7 g/L,  $P < 0.1$ ) and the difference was significant at D30 (+5.2 g/L,  $P < 0.05$ ). In ColG, the total protein remained similar between times but it decreased significantly in ConG ( $P < 0.05$ ). Plasma albumin did not show treatment effect, but values decreased highly significantly with age (4.8 vs 4.2) in ColG and ConG, respectively,  $P < 0.01$ .

The alpha-globulin concentration levels tended to be higher in ColG than in ConG at both sample times (4.6 vs 4.1 and 4.7 vs 4.3, at D10 and D30, respectively,  $P < 0.1$ ). From D10 to D30, changes were not significant. In addition, no effect of interaction was observed.

Plasma beta1 globulin concentrations increased with age (9.9 vs 11.6 g/L in ColG and 8.7 vs 10.5 in ConG, at d10–d30 respectively,  $P < 0.05$ ). No interaction effect was observed but in ConG, the increase tended to be significant (+1.8 g/L,  $P = 0.08$ ). Regarding the change in beta1 globulin from D10 to D30, it was similar between the two groups.

As for beta2 globulin, the mean levels differed significantly between the two groups (3.1 vs 2.3 g/L, in ColG and ConG respectively,  $P < 0.05$ ). Levels remained similar between the two ages, and no interaction effect was observed. Although no group difference was observed at D10, ColG showed higher plasma concentrations than ConG (4.1 vs. 2.2,  $P < 0.05$ ) at D30.

Surprisingly, in the case of plasma gammaglobulin, neither effect of group, time, or interaction, nor that of a contrast could be observed.

Considering the total plasma globulin, only a significant effect of treatment was observed (32.1 vs 29.1 g/L in ColG

**Table 2**

Total and fractions plasma proteins (g/L) at d10 and d30 of age in kids that received or not a supplement of bovine colostrum during the first 15 d of life.

Plasma fractions	D10		D30		Levels of significance				SEM
	ColG (n = 18)	ConG (n = 16)	ColG (n = 18)	ConG (n = 16)	G <sup>1</sup>	T <sup>2</sup>	G × T	Δ <sub>ColG</sub> vs Δ <sub>ConG</sub> <sup>3</sup>	
Total protein	59.8 <sup>aA</sup>	57.1 <sup>aA</sup>	58.1 <sup>aA</sup>	53.2 <sup>bB</sup>	ns†	ns	ns	ns	1.70
Albumin	28.6 <sup>aA</sup>	27.8 <sup>aA</sup>	23.8 <sup>aB</sup>	23.6 <sup>aB</sup>	ns	**	ns	ns	1.40
α-Globulin	4.6	4.1	4.7	4.3	ns†	ns	ns	ns	0.21
β <sub>1</sub> globulin	9.9	8.7 <sup>A</sup>	11.6	10.5 <sup>B</sup>	ns†	*	ns	ns	0.73
β <sub>2</sub> globulin	3.1	2.3	4.1 <sup>a</sup>	2.2 <sup>b</sup>	*	ns	ns	ns	0.50
γ-Globulin	14.5	13.8	13.9	12.0	ns	ns	ns	ns	0.80
Globulin	32.1	29.1	34.3 <sup>a</sup>	29.0 <sup>b</sup>	*	ns	ns	ns	1.72
Alb/Glob	0.91	0.97	0.77	0.90	ns	ns	ns	ns	0.20

a,b Within period treatment effect. For a same period and a same line, values assigned with different uppercase letters are significantly different ( $P < 0.05$ ).

AB Within treatment period effect. For a same treatment and a same line, values assigned with different uppercase letters are significantly different ( $P < 0.05$ ). ns:  $P > 0.05$ ; <sup>1</sup> $P < 0.1$ ; <sup>2</sup> $P < 0.05$ ; <sup>3</sup> $P < 0.01$ .

<sup>1</sup> G: group effect.

<sup>2</sup> T: time effect.

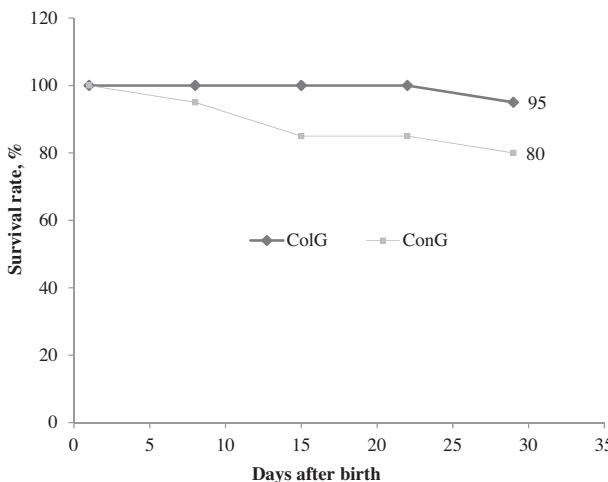
<sup>3</sup> Δ: difference in time within a treatment.

and ConG respectively,  $P < 0.05$ ). The difference between the two groups was significant at D30.

No significant effect on the albumin/globulin ratio was observed.

### 3.2. Growth and survival

**Table 3** presents data on growth characteristics. At birth, the BW of the two groups was similar (1.7 kg). After this period until the end of the trial, the BW increased linearly with time in both group, but with almost twice higher value for the slope of the growth curve in ColG than in ConG ( $P < 0.001$ ), allowing to reach respectively +1.1 kg vs +0.6 kg weight gain at D29 ( $P < 0.001$ ). Within the days, the overall ADG increased until D22 and decreased afterwards. However, the maximum value appeared earlier in ConG (D15) than in ColG (D22). The difference between the two groups strengthened with time (close to 30 g/d at D8 and D15 and 60 g/d at D22 and D29). As a whole, ColG had largely higher ADG than ConG ( $P < 0.001$ ).



**Fig. 2.** Survival rate evolution of kids that received or not a supplement of colostrum during the 15 first day of life. ColG: colostrum group, ConG: control group

**Fig. 2** shows the evolution of survival rate in the two groups. Globally, 5 casualties were recorded, i.e., 1 (5%) in ColG vs 4 (20%) in ConG. Casualties occurred after diarrheal enteritis (25%) and respiratory diseases (75%).

## 4. Discussion

### 4.1. Plasma protein

For total protein, it was first observed a trend for a higher level in ColG at D10, then a significant difference at D30. In addition, statistical analysis showed that neither time nor the interaction ( $G \times T$ ) effects influenced the total plasma protein. Previous studies showed similar effects in the same species (Rodríguez et al., 2009; Moretti et al., 2012). For example, Moretti et al. (2012) found a significant difference in favor to a group of kids which received bovine lyophilized colostrum. According to Mayer et al. (2002), the protein from colostrum, given the first day of life cross the intestinal barrier, following an endocytic process. Presumably, a similar phenomenon occurred in this experiment, but, additionally, an extra-nutritional effect should have operated afterwards and strengthened the differences between the groups. The decrease in plasma total protein with time observed in this experiment is in agreement with results of Lima et al. (2009), who offered bovine colostrum to kids until 60 d of age. The highest concentration in total protein was obtained at 48 h of age and levels decreased thereafter. In another experiment, Lima et al. (2013) did not observe such a decrease with time. In the present trial, the slope of the decrease was weak and similar in the two groups. The trend for an overall difference between the two groups could be ascribed to a positive effect of cow colostrum on plasma protein homeostasis. In addition, plasma protein observed in ColG was much higher (+1.7, +5.8, and +5.4%) than those (57.7, 55.5 and 55.7 g/L) observed by Lima et al. (2013), using either fresh or reconstituted bovine colostrum containing 45–55 mg/mL of IgG, or poor bovine colostrum at 15–25 mg/mL. These results suggest that colostrum from cow is able to maintain high levels of proteins in plasma of kids and that this value is probably similar, and possibly higher, than that obtained with goat colostrum.

**Table 3**

Body weight and ADG (means  $\pm$  standard error) of kids that received or not a supplement of bovine colostrum during the first 15 d of live.

Measurements	Days	Groups <sup>1</sup>		Levels of significance
		ColG	ConG	
BW (kg)	d0	1.7 $\pm$ 0.1 <sup>a</sup>	1.7 $\pm$ 0.1 <sup>a</sup>	ns <sup>2</sup>
	d8	2.6 $\pm$ 0.1 <sup>b</sup>	2.3 $\pm$ 0.1 <sup>b</sup>	
	d15	3.6 $\pm$ 0.1 <sup>c</sup>	3.1 $\pm$ 0.1 <sup>c</sup>	
	d22	4.5 $\pm$ 0.1 <sup>d</sup>	3.6 $\pm$ 0.1 <sup>d</sup>	
	d29	5.6 $\pm$ 0.1 <sup>e</sup>	4.2 $\pm$ 0.1 <sup>e</sup>	
Significance		Group ***	Time ***	Group $\times$ time ***
ADG (g/d)	d8	114.8 $\pm$ 3.8 <sup>a</sup>	80.4 $\pm$ 3.8 <sup>a</sup>	***
	d15	130.3 $\pm$ 3.8 <sup>b</sup>	98.4 $\pm$ 3.9 <sup>b</sup>	
	d22	152.5 $\pm$ 3.8 <sup>c</sup>	90.7 $\pm$ 3.9 <sup>c</sup>	
	d29	144.3 $\pm$ 3.9 <sup>d</sup>	85.7 $\pm$ 3.9 <sup>d</sup>	
Significance		Group ***	Time ***	Group $\times$ time ***

<sup>1</sup> a, b, c, d, e: in the same column, means without common upper-case letter differ ( $P < 0.05$ ).

<sup>2</sup> ns:  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

For albumin, the concentration levels in the both groups significantly decreased between the two periods. Synthesized in the liver, albumin is the most represented protein in plasma, at about 50–65% of total protein (Kaneko et al., 1997). This molecule is essential for the survival of the newborn. Indeed, this protein fraction allows maintaining the oncotic pressure of the blood and transports numerous substances such as fatty acids, calcium, vitamins A and D, antibiotics, steroid hormones (Payne and Payne, 1987). It is dosed to assess the nutritional status of an animal. It is known that in healthy ruminants, changes in serum albumin concentration requires at least one month to be detected, owing to the low turnover – low degradation and synthesis rate – of this protein (Payne and Payne, 1987). Albumin is readily absorbed at the same time as immunoglobulin by the intestinal mucosa of the newborn kid, during the first 24 h of life; Lima et al. (2009) observed that its concentrations decreased after 48 h of age in kids receiving cow colostrum, to reach similar values as to control at 25 d of age. This suggests a higher turnover for albumin from cow. The poor nutritional conditions met in the SCGF could explain the decrease in plasma albumin observed in both groups and the parallel decrease is not surprising.

Regarding globulins ( $\alpha$ ,  $\beta$  and  $\gamma$ ), they are a heterogeneous family of proteins that play a role in the inflammatory response, the transportation of various lipophilic compounds, the homeostasis and the production of antibodies. Although only numerical, the initial higher plasma  $\alpha$ -globulin,  $\beta_1$ ,  $\beta_2$  and  $\gamma$ -globulins levels in ColG, associated with a parallel or higher increase with time when compared to ConG could mean that colostrum provided a competitive advantage to supplemented animals. Ben Romdhane et al. (2001) observed such an increase in camels. Compared to data of literature, the globulin ( $\alpha$ ,  $\beta$  and  $\gamma$ ) levels obtained in this study are higher than those reported by Lima et al. (2013) who obtained less than 20 g/L for the total globulins though their colostrum were richer in globulin than the present one. Data of the present study were in the ranges of 27.0–39.0 g/L interval reported by Kaneko et al. (1997) as indicating a good state of nutrition in domestic animals.

In the case of  $\gamma$ -globulins, differences were not significant. It is not excluded that kids substituted partially the intake of goat colostrum by that of the additional one. It would be worth discriminating in the plasma of kids the immunoglobulin fractions derived from cow and from the mother, and to evaluate their specific half-life. The fall in plasma Ig is physiological. After 24 h, the intestinal barrier loses its capacity to macromolecules internalization and immunoglobulins provide at this stage only local intestinal protection (Moretti et al., 2012). Hadorn and Blum (1997) observed the same effect in calves. Again, Arguello et al. (2004) observed that kids receiving various types of goat colostrum (chill, frozen) expressed maximum levels of Ig at about 24–36 h after birth. Recently Lima et al. (2009) showed that, parallel to total protein, the highest concentration of gamma-globulin in goat kid receiving cow colostrum was obtained at 48 h of age after birth.

Furthermore, it is important to note that the present concentrations of  $\gamma$ -globulins although obtained later, were similar to that obtained before 5 d of age by Moretti et al. (2012) in kids that received a colostrum supplementation.

The results of the present study suggest thus the existence of a positive correlation between colostrum intake and improvement of the plasma protein status.

#### 4.2. Growth and survival

In this study, the significant growth rate difference observed between ColG and ConG could be explained only partly by the intake of supplemental bovine colostrum, because not only these differences increased with time, but also a maximum ADG was observed at the end of the experiment in ColG, by contrast to ConG that expressed the maximum – close to the minimum of the ConG – at the beginning of the experiment. In a previous study, Abdou et al. (2013) observed a highly significant long term – far after the end of the supplementation – difference between the group of animals which were fed colostrum and the control group. In addition, the important increases in BW and ADG recorded all over the present trial in animals receiving bovine colostrum were in agreement with results

of Le Huërou-Luron et al. (2004) and Boudry et al. (2008) who showed the efficacy of bovine colostrum on growth in other species at weaning.

The overall results of this work meet those of Moretti et al. (2010) who indicated a better steady-state of kids after supplemental intake of colostrum. This may be directly associated to the presence of various nutrients in colostrum. Besides providing energy and protein, the supplementation in colostrum probably helped bringing additional levels of vitamins (A and E) and minerals (zinc, selenium and iodine) which gave animals a way to develop and maintain a stronger immunity (Rawal et al., 2008; Morales-delaNuez et al., 2011). Colostrum is also rich in lactoferrin, which plays an important role in defending the body against pathogens (Ajello et al., 2002; Di Mario et al., 2003; Rawal et al., 2008). Indeed, owing to its very strong affinity for iron, it promotes its absorption by the intestinal mucosa of newborns and limits its availability for pathogens (Yamauchi et al., 1998).

In addition, colostrum provides at low concentration polypeptidic compounds such as cytokines (IL-1 $\beta$ , IL-6, IFN-7 and IL-1) belonging to the family of specific and non-specific antimicrobial factors (Hagiwara et al., 2000). In the newborn, these molecules act synergistically with maternal Ig ingested (Playford et al., 2000) and are involved in the regulation of intestinal repair after inflammation (Elson and Beagly, 1994).

No conclusion could be drawn up from survival data, owing to the lack of significant difference between the 2 groups. A previous trial conducted by Mellado et al. (2008) showed that supplementation in colostrum can reduce the mortality rate of youngs. Abdou et al. (2013) studied the survival rate of kids from both groups on a larger period. The mortality rate was highly reduced (5 vs 20% for the ColG and ConG respectively).

## 5. Conclusion and prospect

Our observations suggest that the distribution, early in life, of colostrum from Azawak cow to Red kids is likely to improve their resistance to environmental constraints. This was indicated by higher plasma levels for several protein fractions such as total globulins and total proteins. The supplementation contributed to maintain the plasma protein homeostasis and the effect was preserved at least two weeks after the end of the supplementation. This phenomenon probably helped to improve the healthiness of kids.

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