

## Effects of the administration of *Lactobacilli* on body growth and on the metabolic profile in growing Maltese goat kids

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**Abstract** – The aim of this study was to evaluate the effect of some *lactobacilli* on body growth and on the metabolic-nutritional status in growing goat kids. Twenty growing Maltese goat kids (10 Control and 10 Bios) were studied. The animals of the Bios group received a concentrate including 1 g·kg<sup>-1</sup> of SEB Bovino® (spray-dried), Akron S.r.l., Italy, with non bacterial components: gum arabic, soybean meal, silicate alum of magnesium, and with bacterial components: 10<sup>11</sup> cfu·kg<sup>-1</sup> each of *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus reuteri*. Monthly, bio-metric and weight evaluations were carried out on each animal and individual blood samples were taken. The Bios group showed the highest body weight (Control 19 vs. Bios 23 kg  $P < 0.001$ ), anamorphosis (Control 71 vs. Bios 78  $P < 0.05$ ) and body proportion (Control 35 vs. Bios 41  $P < 0.001$ ) indices; the lowest levels of Non Esterified Fatty Acids (Control 0.778 vs. Bios 0.403 mmol·L<sup>-1</sup>  $P < 0.001$ ), triglycerides (Control 0.21 vs. Bios 0.18 mmol·L<sup>-1</sup>  $P < 0.05$ ), urea (Control 8.83 vs. Bios 7.65 mmol·L<sup>-1</sup>  $P < 0.05$ ) and the highest levels of Alkaline Phosphatase (Control 270 vs. Bios 851 U·L<sup>-1</sup>  $P < 0.01$ ) and Creatine Kinase (Control 173 vs. Bios 285 U·L<sup>-1</sup>  $P < 0.01$ ). The results testify to the better metabolic activity of the Bios group which achieved, at the end of the trial (7 months old), about 99% of the morphological development of the adult, therefore an adequate structure for mating and going into production within the first year of life.

goat kid / growing / *Lactobacillus* / morphological trait / metabolic profile

### 1. INTRODUCTION

The ever growing interest of public opinion and of the lawmaker regarding animal products characterised by high standards of quality and food safety, together with the attention paid to the environment with regards to the quota of polluting substances, have stimulated the research of additives characterised by high bio-availability. Among these

the nutraceutical substances, represented by food or parts of food that are capable of promoting beneficial effects on health, as an alternative to chemical additives [1].

Amongst of nutraceuticals, probiotics are widely used in animal nutrition where they induce favourable changes in the activity of the digestive microflora, with ruminants, the utilisation of probiotics has mainly regarded

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the administration of yeast cultures and Aspergilli in bovines [2–4] such as the administration of strains of *Saccharomyces cerevisiae* in lambs [5].

Lactobacilli are living probiotic non-sporogenic micro-organisms which, added to the feed, provide important benefits to the host animal through the constitution of a healthier and more favourable gastro-enteric setting for digestive and absorption processes [6].

There is abundant literature concerning the effects of lactobacilli, *L. acidophilus*, *L. casei*, *L. bifidus*, and, among these, *acidophilus* is surely the most focalised one on productive performances, on the variation of intestinal flora and on the sanitary state of monogastrics [7, 8].

There is little information on the effects of the administration of lactobacilli in ruminants [9, 10]. McGilliard and Stallings [11] pointed out an increase of the milk yield in cows which received a diet containing lactobacilli and enzymes. Other researchers have shown an improvement in the productive performances and in blood parameters (NEFA,  $\beta$ -OHB, glucose) regarding the energetic metabolism in transition cows [10]. An improvement in the dry matter intake and in body weight, daily gain and fecal score has also been observed in calves during the pre-weaning period [12].

The aim of this study is to evaluate the administration of some lactobacilli on body growth and on the metabolic-nutritional status in goat kids during post-weaning period, in consideration of the following reasons: (i) the proposal of the European Parliament and Council (COM (2002) 153 - C5 - 0143/2002 - 2002/0073 (COD) to reduce strictly the additive use in animal nutrition; (ii) the well-known benefits of the administration of lactobacilli in bovines (cows and calves), especially in particular periods of physiological stress (weaning period, transition period, etc.); (iii) the little information on the nutritional effects of lactobacilli in small ruminant metabolism, particularly in goats.

## 2. MATERIALS AND METHODS

### 2.1. Animals and diets

All goats were treated in accordance with the established standards for use of animals and in full agreement with our local ethical authorities. Twenty growing Maltese goat kids, clinically healthy, at the age of  $45 \pm 5$  days (mean  $\pm$  SD) were divided into two groups of 10, homogeneous for body weight ( $11 \pm 0.1$  kg) and body condition score ( $2.6 \pm 0.12$ ) and raised in two multiple boxes. The trial lasted 150 days, preceded by a 15-day adaptation period. The two groups, called “Control” and “Bios”, received concentrate and meadow hay, twice daily divided into two equal meals at 8.00 and 16.00 hours. The concentrate of the “Bios” group was integrated with  $1 \text{ g}\cdot\text{kg}^{-1}$  of the SEB Bovino<sup>®</sup> (spry-dried), Akron S.r.l., Italy, with non bacterial components: arabic gum, soybean meal, silicate alum of magnesium in addition to the bacterial components: *Lactobacillus acidophilus*  $10^{11}$  cfu $\cdot\text{kg}^{-1}$ , *Lactobacillus salivarius*  $10^{11}$  cfu $\cdot\text{kg}^{-1}$  and *Lactobacillus reuteri*  $10^{11}$  cfu $\cdot\text{kg}^{-1}$ . The ingredients of the concentrates “Ctrl” and “SEB” are reported in Table I. The chemical composition of these feeds (Tab. II) was determined using A.O.A.C. methods [13]. Table III shows the quantities of the feed administered during the experiment.

### 2.2. Body measurements

Once a month, some bio-metric measurements [14], indispensable to calculate the most significant bio-metric indices, were determined on each subject using a Lydtin stick and a flexible meter, with the aim of evaluating the growing performances. The morphologic parameters were studied using 1 linear measurement: height at withers; 1 perimetric measurement: circumference of chest; 2 weight measurements: body weight (BW) using an electronic balance (LAU-MAS Elettronica<sup>®</sup>) and average daily gain

**Table I.** Composition of the concentrates.

Ingredients, (air-dried basis)	“Ctrl” (%)	“SEB” (%)
Corn	35	35.4
Soybean meal (44% CP)	16	16
Barley	14.5	14
Broadbean	10	10
Beat pulp	6	6
Soybean	5	5
Wheat shorts	4	4
Alfalfa meal	4	4
SEB Bovino®	–	0.1
Dicalcium phosphate	1.6	1.6
Sugar cane molasses	1.5	1.5
Calcium carbonate	1	1
Sodium Bicarbonate	0.5	0.5
Vitamins and minerals	0.5	0.5
Salt	0.4	0.4

(ADG). The height at withers was determined on each animal put on a level and hard floor in a natural stance to reduce the possible errors to a minimum. Therefore, two bio-metric indices were calculated to evaluate the proportions among the various parts of the animals' bodies, these being related to the functionality and development of the animals. The calculated indices were volume indices such as the following: anamorphosis index (circumference of chest<sup>2</sup>/height at withers) and body proportion index (body weight/height at withers × 100). Moreover, individual Body Condition Score (BCS) as recommended by Hervieu and Morand-Fehr [15], using a score of 0–5 from the most emaciated to the fattest animal were evaluated as well as individual Fecal Score (FCS) as recommended by Higginbotham and Bath [16], using a score of 1–4 (1: solid/hard faeces; 4: liquid/diarrheic faeces).

**Table II.** Chemical composition of the feed.

	Ctrl Concentrate	SEB Concentrate	Meadow hay
Dry matter (%)	91.90	90.67	91.86
In DM (%)			
Crude Protein	18.55	18.76	11.72
Ether Extract	3.17	3.65	1.88
Non structural carbohydrates	52.61	51.18	20.12
Ash	6.96	7.79	8.89
Neutral detergent fibre	18.71	18.62	57.39
Acid detergent fibre	11.24	11.31	29.05
Acid detergent lignin	2.05	2.03	9.70

**Table III.** Quantity of feed administered (g·head<sup>-1</sup>·d<sup>-1</sup>).

Days	Control group		Bios group		
	Ctrl Concentrate	Meadow hay	SEB Concentrate	Ctrl Concentrate	Meadow Hay
0–30	350	300	350	–	300
31–60	400	400	350	50	400
61–90	400	500	350	50	500
91–120	500	600	350	150	600
121–150	500	700	350	150	700

### 2.3. Blood sampling

Once a month, in the morning while fasting, individual blood samples (10 mL) were taken from the jugular vein into a vacutainer<sup>®</sup> for the evaluation of the metabolic-nutritional status. Blood samples were centrifuged (ALC 4237R) at  $3500 \times g$  for 15 min within two hours of drawing and the sera were frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis [17]. The blood parameters were determined on each individual sample of serum with a spectrophotometer (Lambda 20, Perkin-Elmer) using SIGMA Diagnostics<sup>®</sup> (USA) and RANDOX Laboratories<sup>®</sup> (USA) kits. The following energetic metabolism parameters were determined: Non Esterified Fatty Acids (NEFA,  $\lambda = 550\text{ nm}$ , colorimetric method AAP/TOOS – 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine), Total Cholesterol ( $\lambda = 500\text{ nm}$ , enzymatic colorimetric method CHOD/AAP – cholesterol oxydase and 4-aminoantipyrine), High Density Lipoprotein (HDL,  $\lambda = 500\text{ nm}$ , separation of HDL after precipitation of the LDL and VLDL fractions and determination of the HDL fraction by an enzymatic method), Low Density Lipoprotein (LDL,  $\lambda = 500\text{ nm}$ , precipitation of the LDL at their isoelectric point and determination of the VLDL and HDL fractions in the supernatant by enzymatic methods; LDL value = Total Cholesterol – Cholesterol in the supernatant), Triglycerides ( $\lambda = 500\text{ nm}$ , enzymatic colorimetric method GPO/AAP – glycerol phosphate oxydase and 4-aminoantipyrine), beta-hydroxybutyrate ( $\beta$ -OHB,  $\lambda = 340\text{ nm}$ , enzymatic method: oxidation of  $\beta$ -hydroxybutyrate to acetoacetate). The protein metabolism parameters were the following: total protein ( $\lambda = 546\text{ nm}$  Biuret method), creatinine ( $\lambda = 520\text{ nm}$ , Jaffé method with deproteinisation), urea ( $\lambda = 578\text{ nm}$ , Berthelot reaction); the hepatic functionality parameters were the following: Aspartate aminotransferase (AST,  $\lambda = 365\text{ nm}$ , Optimised Standard Method of the German Society for Clinical Chemistry), alanine aminotransferase (ALT,  $\lambda = 365\text{ nm}$ , Optimised Standard Method of the German

Society for Clinical Chemistry). Finally, the mineral and bone metabolism parameters were the following: alkaline phosphatase (AP,  $\lambda = 405\text{ nm}$ , Optimised Standard Method of the German Society for Clinical Chemistry), creatine kinase (CK,  $\lambda = 365\text{ nm}$ , Optimised Standard Method of the German Society for Clinical Chemistry), calcium (Ca,  $\lambda = 546\text{ nm}$ , o-Cresolphthalein complexone method, without deproteinisation), and inorganic phosphorus (P,  $\lambda = 405\text{ nm}$ , molybdate/vanadate reaction).

### 2.4. Statistical analysis

The data were processed by the GLM procedure of SAS [18] using the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk};$$

where:  $y_{ijk}$  = dependent variable;  $\mu$  = overall mean;  $\alpha_i$  = treatment (Control, Bios);  $\beta_j$  = period (0, 30, 60, 90, 120, 150 days);  $\varepsilon_{ijk}$  = residual error.

## 3. RESULTS

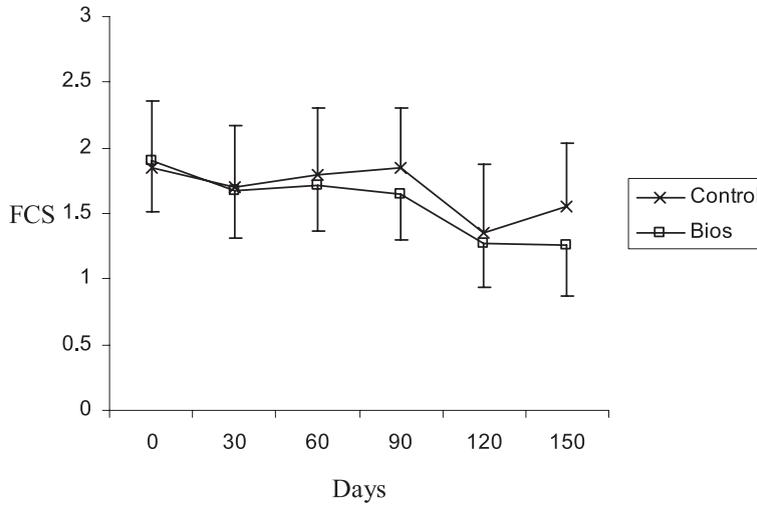
### 3.1. Body measurements

During the trial, the animals of the “Bios” group showed no significant differences for FCS (Fig. 1), significant differences for BW (Fig. 2) and BCS trend (Fig. 3) and a significant difference for ADG at day 60 (Fig. 4).

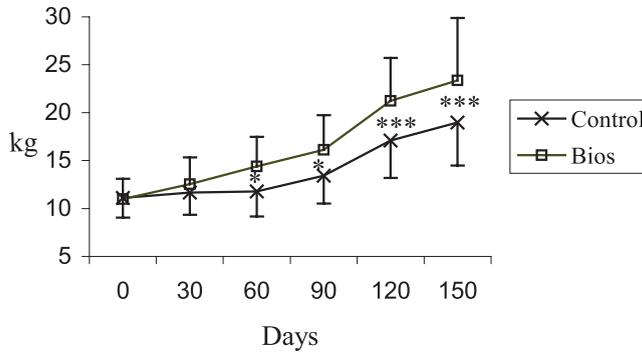
At the end of the trial, the “Bios” group showed a higher circumference of chest and height at withers than the “Control” group (Tab. IV). In confirmation of these results, the “Bios” group had higher anamorphosis ( $P < 0.05$ ) and body proportion ( $P < 0.01$ ) indices (Tab. IV).

### 3.2. Blood parameters

With regards to the parameters concerning energetic metabolism (Tab. V), the “Bios” group showed significant lower levels for NEFA ( $P < 0.001$ ) and for Triglycerides



**Figure 1.** Trend of fecal score (mean ± SD).

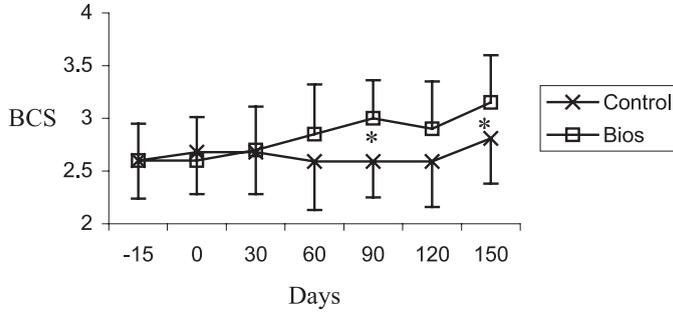


**Figure 2.** Trend of body weight (mean ± SD). \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

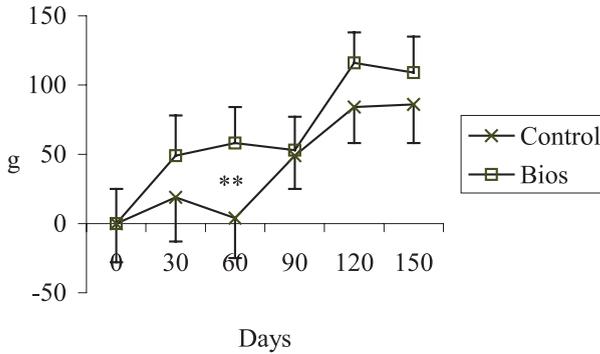
( $P < 0.05$ ) and a higher level for HDL ( $P < 0.001$ ), but no differences were observed for LDL and Total Cholesterol. Among the parameters of protein metabolism, only the urea of the “Bios” group showed significant lower levels ( $P < 0.05$ , Tab. VI). No significant differences were observed for the blood levels of AST and ALT (Tab. VI). Mineral and bone metabolism (Tab. VII) were affected by the treatment, showing during the trial, a significant increase of AP ( $P < 0.001$ ) and CK ( $P < 0.05$ ) in the “Bios” group, while no significant differences were observed for the blood levels of Ca and inorganic P between the two groups (Tab. VII).

#### 4. DISCUSSION

The animals of the “Bios” group showed better growth performances testified by a higher BW, by a higher anamorphosis index, which resulted from a greater development of the respiratory and gastro-intestinal apparatus, and by a higher body proportion index, which resulted from a better development of the skeletal structure in a longitudinal direction, which is an important characteristic considering the milk-producing aptitude of the Maltese goat. These results are in agreement with the research carried out on growing calves which received  $15 \text{ g} \cdot \text{head}^{-1}$



**Figure 3.** Trend of body condition score (mean ± SD). \*  $P < 0.05$ .



**Figure 4.** Trend of average daily gain (mean ± SD). \*\*  $P < 0.01$ .

**Table IV.** Body measurements and biometric indices at the end of the trial (mean ± SE).

	Control	Bios	<i>P</i>
Body Weight (kg)	18.97 ± 0.80	23.37 ± 0.84	< 0.001
Circumference of chest (cm)	62.09 ± 1.13	66.60 ± 1.18	0.007
Height at withers (cm)	54.27 ± 0.77	57.20 ± 0.812	0.0103
Anamorphosis index	71.21 ± 2.02	77.71 ± 2.12	0.028
Body proportion index	34.85 ± 1.30	40.77 ± 1.36	0.002

of a pool of lactobacilli, at birth and at 7 days [19].

The BCS trend and the significant lower levels of NEFA and Triglycerides in the “Bios” group, showed a better metabolic status and a positive energetic balance of the animals of this group in agreement with the findings of Savoini et al. [10] on transi-

tion cows that received 15 g·head<sup>-1</sup> of a pool of 3 lactobacilli, at 10 and 4 days before birth and at 1 and 2 days after calving. The higher levels of Total Cholesterol, LDL and the significant higher level of HDL in the “Bios” group, were probably correlated to a greater intestinal absorption of medium and long chain fatty acids, which would be

**Table V.** Energetic metabolism parameters (mean  $\pm$  SE).

mmol·L <sup>-1</sup>	Control	Bios	<i>P</i>
NEFA	0.778 $\pm$ 0.06	0.403 $\pm$ 0.06	< 0.001
Triglycerides	0.21 $\pm$ 0.01	0.18 $\pm$ 0.01	0.022
Total Cholesterol	1.50 $\pm$ 0.08	1.69 $\pm$ 0.08	0.072
HDL	0.81 $\pm$ 0.02	0.93 $\pm$ 0.02	< 0.001
LDL	0.60 $\pm$ 0.06	0.68 $\pm$ 0.06	0.367
$\beta$ -OHB	3.40 $\pm$ 1.40	5.10 $\pm$ 1.40	0.431

**Table VI.** Protein metabolism parameters and hepatic functionality (mean  $\pm$  SE).

	Control	Bios	<i>P</i>
Total protein (g·L <sup>-1</sup> )	5.90 $\pm$ 0.06	5.80 $\pm$ 0.06	0.933
Urea (mmol·L <sup>-1</sup> )	8.83 $\pm$ 0.38	7.65 $\pm$ 0.38	0.037
Creatinine ( $\mu$ mol·L <sup>-1</sup> )	89.28 $\pm$ 0.88	79.56 $\pm$ 0.88	0.359
AST (U·L <sup>-1</sup> )	74.50 $\pm$ 2.30	71.70 $\pm$ 2.30	0.399
ALT (U·L <sup>-1</sup> )	18.40 $\pm$ 0.60	20.30 $\pm$ 0.60	0.055

**Table VII.** Mineral and bone metabolism parameters (mean  $\pm$  SE).

	Control	Bios	<i>P</i>
AP (U·L <sup>-1</sup> )	270.40 $\pm$ 89.00	851.00 $\pm$ 97.00	< 0.001
CK (U·L <sup>-1</sup> )	172.70 $\pm$ 31.50	285.30 $\pm$ 31.50	0.015
Ca (mmol·L <sup>-1</sup> )	2.20 $\pm$ 0.02	2.18 $\pm$ 0.02	0.633
P inorganic (mmol·L <sup>-1</sup> )	2.59 $\pm$ 0.13	2.54 $\pm$ 0.13	0.793

esterified in situ and introduced again as lipoproteins (Total Cholesterol, LDL, HDL) and chylomicrons into the blood [17], testifying to an improvement in the intestinal absorption of the nutrients for the treated group.

With regards to the protein metabolism, the significant lower content of urea in the “Bios” group could be justified by the better nutritional status of these animals that do not resort to the amino acid deamination [20, 21] in order to obtain energy.

The significant higher levels of AP and CK in the “Bios” group, the former correlated to a higher osteoblastic activity and so to a greater skeletal development [22] and

the latter to a muscular development [23], were confirmed by the calculated bio-metric indices.

Some hypotheses on the possible mode of action of the lactobacilli could be proposed. The administration of lactobacilli and their distribution on the surface of the digestive tract [8] could have (1) positively influenced the development and the preservation of the fermentative rumen activity, improving the utilisation of the feed and the dry matter intake; (2) determined the conditions of optimal pH for the activity of pancreatic enzymes [10], leading to an improvement of the intestinal absorption of the nutrients; (3) reduced the number and activity of the

proteolytic micro-organisms, improving the trophism of the intestinal mucosa in a secreting and absorbing way [24].

## 5. CONCLUSIONS

The administration of lactobacilli could represent an interesting natural alimentary product for the protection and for the support of the growing performances and the metabolic-nutritional status of animals, inducing favourable changes in the activity of the digestive microflora, positively influencing the development and the preservation of the fermentative rumen activity, improving the utilisation of the alimentary fractions of the diet and the dry matter intake. These observations represent some of the most important requirements to assure a right productive and reproductive efficiency of the animal. These were confirmed by the experimental subjects fed on lactobacilli which achieved, at the end of the trial (7 months old), about 99% of the morphological development of the adult [25]. This means that the animals had reached an adequate structure ready for mating and going into production within the 1st year of life, in line with the expectations of the breeder and at the same time respecting animal welfare and environmental protection.

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