Effect of probiotic supplementation on growth performance, nutrient utilization and carcass characteristics of growing Chinchilla rabbits

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1. Introduction
Weaning in rabbits is a complex step causing dietary, environmental and psychological stresses influencing feed intake, gastro-intestinal (GI) tract development and adaptation to the weaning diet. A period of 10–15 d after weaning is the most critical period when rabbits are most susceptible to GI infections and at greatest risk of a fatal outcome (Gallois et al. 2008; Bivolarski & Vachkova 2013). Due to the unique physiology of digestive tract, rabbits usually show a fragile balance in their gut function and frequently suffer from post-weaning alimentary disturbances and probiotics may contribute to improve their health status (Trocino et al. 2005; Kritas et al. 2008). Probiotics are defined as ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance’ (Fuller 1989). Probiotics can influence GI weight and also the microbial fermentation pattern in the caecum (Kermauer & Struklec 1999). Falcao et al. (2007) reviewed results of probiotics in rabbits reporting a positive effect on average daily gain (ADG), feed conversion ratio and a lower mortality in most of the experiments. Yu and Tsen (1993) reported that as Lactobacillus are not the regular inhabitants of rabbit digestive tract, Lactobacillus-based probiotics may not be useful in rabbits.

Based on the information an experiment was planned to study the comparative efficacy of two probiotic cultures on growth performance, nutrient utilization and carcass traits in weaner rabbits.

2. Material and methods
The experiment was carried out at the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India). The study was initiated in March 2012 and ended in May 2012. During the experiment, minimum and maximum ambient temperature ranged from 7.5°C to 18.9°C and 39.0°C to 45.5°C, respectively, and relative humidity varied from 17.0% to 76.0%. The animal care, handling and sampling procedures were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animal, India.

Forty-five Chinchilla (mixed sex) weaner rabbits (42-d-old, average body weight (BW) 619 ± 19.5 g) were randomly distributed in three equal groups consisting of 15 rabbits per group. The dietary treatments were control (no probiotic), while the other two groups were supplemented with probiotics (10^7 CFU/g concentrate) Lactobacillus acidophilus (Lo) and Lactococcus lactis (Ll), respectively. The rabbits were kept individually under similar housing and management conditions in wire mesh cages (180 × 240 × 180 mm) inside asbestos-roofed animal shed and were fed ad libitum mash (ground to 2 mm diameter) concentrate mixture (maize 31, barley 31, groundnut cake 31, molasses 5, mineral mixture 1.5 and NaCl 0.5 kg/100 kg) and wilted green Lucerne (Medicago sativa, Table 1) in two separate feeders. Probiotic culture of Lo and Ll was prepared in the laboratory from pure slants of these microbes revived on 5.5% MRS (Man Rogosa Sharpe) broth and cultured freshly every day in 1.2% skimmed milk for 24 h. Ten mL of cultured
The liver, heart with lung, periscapular and perirenal weighed, including the contents to arrive at full GI tract. Gastrointestinal tract and caecum were separated and weighed. The remaining part was considered as hot carcass. Dressing-out% was calculated as hot carcass weight divided by pSLW × 100. Hot carcasses were chilled at 4°C for 24 h to arrive at chilled carcass weight. A sample of Longissimus dorsi muscle was taken for estimating nutrient composition (AOAC 2000) and fatty acid profile (Bhatt et al. 2015). Briefly, the meat samples are homogenized and processed employing the alkaline hydrolysis method (Murrieta et al. 2003) to obtain fatty acid methyl ester, which were injected into gas chromatograph (DANI Master GC, Series 100922002, Italy) in fused silica capillary column (SPTM-2560, 100 m 9 0.25 mm 9 0.1 mm thickness) fitted to a PTV injector and FID. The analytical conditions were injection port temperature 220°C, column temperature step up from 170°C to 240°C in 53 min and detector temperature 250°C with flow rate (mL/min) of carrier gas nitrogen 30, hydrogen 40 and air 280.

Data were subjected to analysis of variance using SPSS Base14.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, IL 60606-6307, USA) and by fitting into the mathematical model:

\[ Y_{ijk} = \mu + T_i + e_{ijk}, \]

where \( Y_{ijk} \) = observation mean; \( \mu \) = general mean, \( T_i \) = effect of \( i \)th treatment for \( j \)th observation and \( e_{ijk} \) = random error. A repeated measure analysis was employed for assessing periodic growth performance. Significant differences among the mean values for the three dietary treatments were tested using Duncan’s Multiple Range Test.

### 3. Results and discussion

The CP content of concentrate mixture was 20.8%, while that of and the Lucerne was low at 11.6% (Table 1). The green Lucerne was wilted (8 am to 3 pm) and then offered to rabbits. It contained 40.1% DM and was relatively mature that contained a very low CP (ICAR 2013). Meanwhile, an appreciable DM intake (40 g/d; Table 2) of Lucerne confirmed its acceptability and palatability by the rabbits in all the dietary groups.

The growth performance of rabbits in different dietary groups was assessed at 56, 77 and 91 d of age. Rabbits in the La group cumulated higher BW than the control and the Li had intermediate BW (Table 2). The ADG showed an interesting pattern with significantly (\( P < .05 \)) higher values in La than the control when assessed for the periods 43–56 d, 57–77 d and overall (43–91 d) while it was non-significant during 78–91 d of age. The rabbits in Li group had significantly higher ADG than the control only during 43–56 d of age. There was no significant difference in ADG between the probiotic supplemented groups. The feed intake was not affected due to probiotic supplementation and with the result, the La and Li-fed rabbits showed better feed conversion efficiency (FCR) in rabbits supplemented with probiotics containing B. licheniformis and B. subtilis. In an earlier study, Kama et al. (1996) reported no effect of probiotic (Lactobacillus acidophilus) supplementation on dry matter intake and growth of rabbits. The efficacy of a commercial probiotics (BioPlus 2B®; B. licheniformis, B. subtilis) on weekly growth

### Table 1. Chemical composition (% on dry matter basis) of concentrate and lucerne fed to rabbits.

| Component         | Concentrate | Lucerne |
|-------------------|-------------|---------|
| Dry matter        | 96.0        | 40.1    |
| Organic matter    | 91.5        | 91.9    |
| Crude protein     | 20.8        | 11.6    |
| Ether extract     | 2.3         | 2.3     |
| Total ash         | 8.5         | 8.1     |
| Neutral detergent fibre | 28.2 | 53.5 |
| Acid detergent fibre | 8.7 | 34.0   |
| Hemicellulose     | 19.1        | 19.5    |
| Cellulose         | 7.3         | 19.0    |
| Acid detergent lignin | 7.3 | 15.0   |

aConcentrate consisted of 31% maize, 31% barley, 31% groundnut cake, 5% molasses, 1.5% mineral mixture (g/kg: dicalcium phosphate 537, calcium carbonate 42.5, manganese chloride 9.72, zinc sulphate heptahydrate 17.1, ferrous sulphate heptahydrate 4.98, and copper sulphate pentahydrate 1.18, starch powder 567.5) and 0.5% NaCl.
bLucerne fed to rabbits was in green wilted form (cut at 8 am and offered at 3 pm daily).
the use of antibiotics and growth promoters in animal feeds seeks for new lines of products, more acceptable to the consumer, most preferably the use of probiotics and prebiotics aimed at reducing the incidence of enteric diseases and to improve feed intake and digestibility (Mateos et al. 2010).

A higher growth rate coupled with improved FCR might be accounted for the differences observed in the faecal digestibility (Table 3). The digestibility of DM and organic matter (OM) improved with the supplementation of La compared to the control diet (72.6 vs. 66.8%. P < .05), whereas rabbits fed with Li showed an intermediate value. Also, supplementation of both La and Li effected improvement in digestibility of crude protein (P = .001), NDF (P = .008) and hemicelluloses (NDF-ADF) than the control. El-Hindawy et al. (1993) observed improvement in the digestibility of all the nutrients when probiotic Lacto-Sacc (containing Lactobacillus acidophilus, Streptococcus faecium, Saccharomyces cerevisiae and fermentation residues) was added. Kamra et al. (1996) observed an improved CP digestibility and Yamani et al. (1992) reported higher crude fibre digestibility in rabbits supplemented with similar probiotics. Amber et al. (2004), working with Lactobacillus acidophilus, got improvements in the digestibilities of energy and of most analytical fractions (DM, CP and EE), including crude fibre. A non-significant effect on digestibility was also observed by some researchers (Gippert et al. 1992; Luicke et al. 1992) upon La and Li supplementation in pelleted diet to rabbits. Looking at the beneficial effects of probiotics, maintenance of GI health and stimulation of enzyme production by the host performance of rabbits found similar feed intake and BW gain with non-significant difference in FCR (Kustos et al. 2004; Matusevicius et al. 2006). Nonetheless, they found it advantageous to supplement the diet of growing rabbits in summer conditions, primarily aiming to reduce the sanitary risk during the fattening period. Weaner rabbits are generally exposed to stress of nutritional alterations besides environmental and social stresses being away from its mother. Nutritional alterations, in particular, cause microflora disturbances at the level of intestine and disrupt local immunological mechanisms, leading to increased frequency of enteric diseases (O’Hara & Shanahan 2006). It has been shown that feeding of probiotics may have a growth promoting activity by competing with harmful gut flora and stimulating the immune system (Kritas et al. 2008). Although data on probiotics feeding in rabbits are a few when compared to species such as pigs and poultry, Falcao-e-Cunha et al. (2007) reviewed the results published in the literature and discussed possible mechanisms of action on digestibility and caecal activity. Copeland et al. (2009) found probiotic fortified diet effective in decreasing pathogenic bacteria colonization in a long-term neonatal rabbit model. They did not find any difference in BW gain between control and probiotic-fed groups. When probiotics are used aiming to replace the antibiotics in the diet, the zoo technical traits are scarcely improved but with promoting the development and maintenance of the caecal flora they result in good health status of the animals. A higher growth rate in probiotic supplemented rabbits may also suggest a better health status, more importantly the GI health of the rabbits (Falco-e-Cunha et al. 2007; Kritas et al. 2008). A significant difference in growth pattern between control and La-supplemented rabbits during 43–77 d of age may thus be attributed to the maintenance of improved gut health that could ameliorate early weaning stress. The rabbits in the control group gradually adapted to nutritional and environmental alterations and showed compensatory BW gain from 18.8 (43–56 d) to 22.9 g during 57–77 d and 23.4 g during 78–91 d of age. The rabbits supplemented with probiotics Li showed an intermediate and steady growth performance during the whole period. The prevailing uncertainty regarding

### Table 2. Effect of probiotic supplementation on growth performance of rabbits.

|                | Control | Lactobacillus acidophilus | Lactococcus lactis | SEM  | P-value |
|----------------|---------|---------------------------|--------------------|------|---------|
| Initial body weight (42 d, g) | 622     | 620                       | 627                | 34.6 | 0.841   |
| Body weight (56 d, g) | 886     | 941                       | 955                | 42.4 | 0.415   |
| Body weight (77 d, g) | 1360    | 1473                      | 1452               | 46.1 | 0.118   |
| Final body weight (91 d, g) | 1678a   | 1821b                     | 1776ab             | 47.4 | 0.047   |
| Feed intake, g DM/d |         |                           |                    |      |         |
| Feed conversion ratio, g/g | 4.34a   | 3.91b                     | 3.96b              | 0.065| 0.009   |

Notes: SEM, standard error of mean (n = 15). Values with different superscripts in a row differ significantly (P < .05).

*Calculated as g total DM intake per g weight gain.

### Table 3. Effect of probiotic supplementation on total tract apparent digestibility of nutrients and nitrogen balance in rabbits (n = 5).

| Attributes            | Control | Lactobacillus acidophilus | Lactococcus lactis | SEM   | P-value |
|-----------------------|---------|---------------------------|--------------------|-------|---------|
| Dry matter intake (g/day) |         |                           |                    |       |         |
| Concentrate           | 55.0    | 57.9                      | 53.9               | 4.57  | 0.49    |
| Lucerne               | 45.6    | 46.1                      | 46.1               | 1.50  | 0.95    |
| Apparent digestibility (%) |       |                           |                    |       |         |
| Dry matter            | 67.0a   | 72.8b                     | 70.0ab             | 1.51  | 0.040   |
| Organic matter        | 66.6a   | 72.4b                     | 69.0ab             | 1.49  | 0.044   |
| Crude protein         | 74.9a   | 82.7b                     | 82.6b              | 1.04  | 0.001   |
| Ether extract         | 76.6    | 72.2                      | 74.1               | 1.61  | 0.21    |
| Neutral detergent fibre (NDF) | 29.4a | 40.9b                     | 42.8b              | 2.67  | 0.008   |
| Acid detergent fibre (ADF) | 7.5   | 11.9                      | 8.5                | 1.53  | 0.15    |
| NDF-ADF (Hemicellulose) | 23.4a | 30.9b                     | 30.4b              | 1.66  | 0.016   |
| ADF-ADL (Cellulose)   | 66.8    | 70.0                      | 66.0               | 2.01  | 0.36    |
| Nitrogen (N) balance (g/day) |       |                           |                    |       |         |
| N intake              | 2.83    | 3.03                      | 2.84               | 0.148 | 0.56    |
| N excretion through faeces | 0.71a | 0.52b                     | 0.49b              | 0.042 | 0.007   |
| N excretion through urine | 0.73   | 0.77                      | 0.70               | 0.082 | 0.753   |
| N absorbed            | 2.12a   | 2.51b                     | 2.35ab             | 0.104 | 0.027   |
| N retained            | 1.39a   | 1.74b                     | 1.64ab             | 0.099 | 0.034   |
| N retention (% of intake) | 48.6a | 57.5b                     | 57.5b              | 2.13  | 0.044   |
| N retention (% of absorbed) | 74.7a | 82.8b                     | 82.5b              | 2.65  | 0.047   |

Notes: SEM, Standard error of mean. Values with different superscripts in a row differ significantly (P < .05).
(Mateos et al. 2010), may possibly contributed towards improvement in nutrient digestibility in probiotic-supplemented groups. Concomitant with an improvement in CP digestibility, the N balance parameters showed higher retention ($P = .034$) based on N economy through reduced faecal excretion ($P = .007$) in probiotic-supplemented groups than the control while urinary N excretion remained unaltered (Table 3). The utilization pattern of N as a percentage of intake and absorbed followed a similar trend. The N balance data correlated well with ADG in different groups. Gastrointestinal pathogenesis incurs nutrient drainage from the body especially that of protein due to endogenous catabolism and mucus secretion as a part of maintenance activity in the event of infection (Coop & Kyriazakis 1999) and/or inflammation, malabsorption and metabolic rearrangement aimed at local tissue repair. It is thought that probiotics supplements colonize the gut, contributing to the maintenance of the flora equilibrium that ultimately provides a gut barrier against pathogens (Mateos et al. 2010). A higher digestibility of CP and fibre components (NDF, hemicelluloses) in probiotics-supplemented groups could be the result of maintaining a relatively better GI health and gut environment that supported improvement in N utilization and growth with efficient FCR. Rabbits consumed nearly 42% of total DM intake from relatively mature Lucerne which emphasized utilization of low-cost fibrous feeds, thus contributing to improved nutrient accretion from higher fibre digestibility in probiotics-supplemented groups. Fermentation metabolites, particularly volatile fatty acids production can cover 30–50% of maintenance energy requirements of adult rabbits (Combes et al. 2012). Moreover, a higher digestibility/fermentation of hemicelluloses in the lower GI tract might also have aided the maintenance of acidic pH to act against pathogenic microorganisms and supporting gut health. This prebiotic function in conjunction with probiotics would be of definite advantages to the host thereby delivering a symbiotic effect.

No significant effect of probiotic supplementation was observed on carcass traits (Table 4). Rabbits in all dietary groups had similar dressing yield (average 58%), which corroborate well with the earlier reported values (Marounek et al. 2007; Rotolo et al. 2014). Besides, a measure of caecum weight and the epithelial layer thickness were assessed, which revealed relatively higher weight and a significantly increased thickness in both the probiotics-supplemented groups than the control. De Blas et al. (1991) observed a heavier caecum in weanling rabbits upon dietary inclusion of a probiotic (Paci®). Some authors reported that probiotics can influence GI weight and the relative proportion of its organs and also the microbial fermentation pattern in the caecum (Kermansa & Strukle 1996; Kermansa et al. 1996). Rotolo et al. (2014) observed similar caecum weight as percentage of total BW. Proliferation of colonicocytes in response to prebiotic effects of hemicelluloses and colonization of supplemental probiotics might have contributed to thickening of mucosa without hindering the nutrient absorption process.

Rabbit meat offers excellent nutritive and dietetic properties (Hernández & Gondret 2006; Zotte & Szendrő 2011). There was no significant effect ($P > .05$) of probiotic supplementation on proximate composition and fatty acid profile of Longissimus dorsi muscle (Table 5). The findings are in agreement with those of Combes and Dalle Zotte (2005) and higher than the values of CP content reported by Bhatt et al. (1996) and Simonová et al. (2009). Similarly, the fatty acid profile of LD muscle revealed non-significant effect of probiotics supplementation. The saturated fatty acid (SFA) content at 37.5% with a PUFA: SFA ratio at 1.06 emphasized qualitative and dietetic properties of rabbit meat. The results on fatty acid profile are in line with the earlier reported values (Jorge et al. 2005; Marounek et al. 2007). Nonetheless, the chemical composition and fatty acid profile fell within the normal range for rabbit meat (Zotte & Szendrő 2011). Simonová et al. (2009) did not also observe any negative influence of application of bacteriocinogenic and probiotic strain on rabbit meat quality and nutritional value. Zhang et al. (2010) outlined three main ways of improving meat and meat product functional value: (1) by adding

| Parameter                  | Control | Lactobacillus acidophillus | Lactococcus lactis | SEM | $P$-value |
|----------------------------|---------|---------------------------|-------------------|-----|----------|
| Pre-slaughter weight (g)   | 2121    | 2110                      | 2101              | 39.7 | 0.835    |
| Dressed weight (g)         | 1235    | 1203                      | 1235              | 24.8 | 0.860    |
| Head weight (g)            | 186     | 181                       | 185               | 3.1  | 0.788    |
| Skin weight (g)            | 202     | 196                       | 205               | 7.2  | 0.804    |
| Kidney, heart and lung weight (g) | 76.0 | 71.0                      | 68.0              | 2.74 | 0.328    |
| Dressing percent (%)       | 58.2    | 57.0                      | 58.8              | 1.17 | 0.677    |
| Cecum weight (g)           | 113.0   | 129.0                     | 109.0             | 8.27 | 0.415    |
| Cecum weight (% of live weight) | 5.38 | 6.17                      | 5.18              | 0.42 | 0.267    |
| Epithelial layer thickness (mm) | 0.44<sup>a</sup> | 0.61<sup>b</sup> | 0.63<sup>c</sup> | 0.03 | 0.023 |

Notes: SEM, Standard error of mean ($n = 5$). Values with different superscripts in a row differ significantly ($P < .05$).

| Parameter                  | Control | Lactobacillus acidophillus | Lactococcus lactis | SEM | $P$-value |
|----------------------------|---------|---------------------------|-------------------|-----|----------|
| Proximate composition (%)  |         |                           |                   |     |          |
| Water                      | 71.4    | 71.5                      | 71.6              | 0.46 | 0.945    |
| Dry matter                 | 28.5    | 28.5                      | 28.4              | 0.16 | 0.870    |
| Organic matter             | 26.8    | 26.9                      | 26.7              | 0.18 | 0.908    |
| Crude protein              | 25.5    | 25.7                      | 25.5              | 0.23 | 0.929    |
| Fat                        | 1.28    | 1.28                      | 1.07              | 0.084 | 0.678    |
| Mineral                    | 1.74    | 1.54                      | 1.76              | 0.091 | 0.457    |
| Fatty acid profile (% of total fatty acids) |         |                           |                   |     |          |
| Myristic                   | 1.28    | 1.70                      | 1.54              | 0.188 | 0.344    |
| Palmitic                   | 21.2    | 22.8                      | 19.6              | 1.13 | 0.267    |
| Saturated                 | 9.2     | 10.0                      | 10.9              | 0.88 | 0.763    |
| Oleic acid                 | 13.6    | 12.1                      | 12.7              | 1.04 | 0.612    |
| Linoleic acid              | 32.4    | 32.8                      | 32.8              | 2.32 | 0.988    |
| Linolenic acid             | 3.55    | 3.59                      | 3.59              | 0.162 | 0.977    |
| Arachidonic acid           | 3.45    | 3.60                      | 3.55              | 0.098 | 0.518    |
| SFA                        | 37.8    | 39.7                      | 35.2              | 2.34 | 0.784    |
| MUFA                       | 20.8    | 21.1                      | 22.1              | 2.15 | 0.487    |
| PUFA                       | 39.4    | 40.0                      | 39.9              | 1.69 | 0.740    |
| PUFA/SFA                   | 1.04    | 1.01                      | 1.13              | 0.03 | 0.575    |
| 18:2/18:3                  | 9.12    | 9.14                      | 9.12              | 0.18 | 0.912    |

Notes: SEM, Standard error of mean ($n = 5$). Values with different superscripts in a row differ significantly ($P < .05$).
functional compounds to animal diets; (2) by incorporating functional ingredients, including probiotics and acid lactic bacteria, into meat products during processing and (3) by favouring the production of functional components during processing and enzymatic hydrolysis. The effect of probiotics on enhanced nutrient digestibility (especially the fibre components, hemicelluloses), N utilization, growth and FCR in conjunction with maintenance of better gut morphology may have futuristic implication while undertaking dietary modulation of rabbit meat quality.

4. Conclusion

It may be concluded that probiotics supplementation, more specifically Lactobacillus acidophilus (at 10^7 CFU/g concentrate) improved digestibility and utilization of nutrients, BW gain and feed conversion ratio with apparently no significant changes in carcass traits, composition and fatty acid profile. Moreover, improved digestive efficiency through optimization of the composition of the gut microbiota has a direct impact on feed costs, and would also increase the use of low-cost fibrous feed.

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No potential conflict of interest was reported by the authors.

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