Title: Effect of Different Protein Levels with or without Probiotics on Growth Performance and Blood Metabolite Responses Pre and Post Weaning in Male Kurdi Lambs

Abstract: Two experiments were conducted to investigate the effects of different protein levels with or without probiotics in pre and post weaning phase on growth performance, blood metabolites and rumen fermentation parameters of male fat-tailed Kurdi Lambs. In Experiment 1 (pre-weaning phase), twenty-four 30-d-old lambs with an average body weight (15.25±1.8 kg) were randomly divided into four groups and individually fed in a 2 × 2 factorial arrangement of treatments. This experiment lasted 67 days, including 15 days feed adjustment period and fed their respective rations until weaning time (45 days of experiment) and 7 day after weaning. Four diets were formulated using two protein levels (16% and 18% CP) with (2g /day) or without probiotics. The results of the experiment 1 showed that starter conversion ratio, daily gain, blood metabolites except cortisol and rumen pH was not affected (P<0.05) in pre-weaning phase in lambs fed diets containing different protein levels. Starter intake was highest (P<0.05) in lambs fed a diet containing 18% CP and probiotics than other treatments. Addition of probiotics did not influence (P<0.05) the starter conversion ratio, daily gain and some blood metabolites (glucose, triglyceride and total protein) in experiment 1. Blood urea nitrogen and serum minerals remained unaffected (P<0.05) in lambs fed diet containing different protein sources. No difference (P<0.05) was noticed in blood cortisol concentration across all the protein level, but blood cortisol concentration was affected due to probiotics supplementation. Increased blood cortisol concentrations were observed at 24h after weaning and were maintained 48 h after weaning. In addition, probiotics supplementation was significantly (P<0.05) reduced blood cortisol concentrations in lambs. The results of the experiment 2 showed that feed intake, feed conversion ratio and daily gain was similar (P>0.05) in post-weaning phase in across all the treatments. Ruminal pH and ammonia-N concentration were significantly higher (P < 0.05) for probiotics supplemented. However, lambs fed 16.5% CP diets showed higher BUN than those fed 14.5% CP diets and the same was true for blood
These results indicate that supplementation with probiotics can improve the starter intake and reduce blood cortisol concentration during the weaning phase in lambs.
Effect of Different Protein Levels with or without Probiotics on Growth Performance and Blood Metabolite Responses Pre and Post Weaning in Male Kurdi Lambs

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Abstract
Two experiments were conducted to investigate the effects of different protein levels with or without probiotics in pre and post weaning phase on growth performance, blood metabolites and rumen fermentation parameters of male fat-tailed Kurdi Lambs. In Experiment 1 (pre-weaning phase), twenty-four 30-d-old lambs with an average body weight (15.25±1.8 kg) were randomly divided into four groups and individually fed in a 2×2 factorial arrangement of treatments. This experiment lasted 67 days, including 15 days feed adjustment period and fed their respective rations until weaning time (45 days of experiment) and 7 day after weaning. Four diets were formulated using two protein levels (16% and 18%
CP) with (2g/day) or without probiotics. The experimental procedure in experiment 2 (post-
weaning phase) was essentially the same as that for experiment 1 with minor exceptions.
Twenty-four nine months were randomly divided into four groups in a $2 \times 2$ factorial
arrangement of treatments. This experiment lasted 60 days, including 15 days feed
adjustment period and fed their respective rations until sampling. Four diets were formulated
using two protein levels (14.5% and 16.5% CP) with (2g/day) or without probiotics. The
results of the experiment 1 showed that starter conversion ratio, daily gain, blood metabolites
except cortisol and rumen pH was not affected (P<0.05) in pre-weaning phase in lambs fed
diets containing different protein levels. Starter intake was highest (P<0.05) in lambs fed a
diet containing 18% CP and probiotics than other treatments. Addition of probiotics did not
influence (P<0.05) the starter conversion ratio, daily gain and some blood metabolites
(glucose, triglyceride and total protein) in experiment 1. Blood urea nitrogen and serum
minerals remained unaffected (P<0.05) in lambs fed diet containing different protein sources.
No difference (P<0.05) was noticed in blood cortisol concentration across all the protein
level, but blood cortisol concentration was affected due to probiotics supplementation.
Increased blood cortisol concentrations were observed at 24h after weaning and were
maintained 48 h after weaning. In addition, probiotics supplementation was significantly
(P<0.05) reduced blood cortisol concentrations in lambs. The results of the experiment 2
showed that feed intake, feed conversion ratio and daily gain was similar (P>0.05) in post-
weaning phase in across all the treatments. Ruminal pH and ammonia-N concentration were
significantly higher (P < 0.05) for probiotics supplemented. However, lambs fed 16.5% CP
diets showed higher BUN than those fed 14.5% CP diets and the same was true for blood
triglyceride. These results indicate that supplementation with probiotics can improve the
starter intake and reduce blood cortisol concentration during the weaning phase in lambs.
Key Words: Protein, Probiotics, Kurdi Lambs, Pre-Weaning, Growth Performance

1. Introduction

Many studies have focused on improving the efficiency of feed utilization for various production goals; especially in regions of the world where ruminants are fed low quality feed protein. The use of feed additives like probiotics is one of the strategies to improve the efficiency of microbial protein synthesis in the rumen (Mwenya et al., 2004), and the N-retention by enhancing microbial peptidolytic and proteolytic activities in the rumen (Cole et al., 1992) and stimulate desirable microbial growth in the rumen, and accelerates weaning. The addition of probiotics in feeding systems has been shown to improve live-weight gain in calves following weaning and stimulated rumen development in calves during weaning (Theodorou et al. 1990).

It is therefore obvious that there is an increasing consumer concern about the long-term effects of antibiotics has led to a more focused interest in research in the use of probiotics instead of ionophores and antibiotics as manipulators of rumen fermentation to improve animal performance and ruminal function (Chaucheyras-Durand et al., 2010). A probiotic is defined classically as a live microbial dietary supplement that beneficially affects the host animal by improving its intestinal microbial balance of the host animal (Cruywagen et al., 1996). Probiotics supplementation improves the microbial activities in rumen resulting in enhanced NH$_3$ capture to synthesize microbial protein (Erasmus et al., 1992).

Protein appropriate levels in livestock feed are necessary for optimum microbial growth and protein synthesis which might otherwise result in the wastage of large amounts of nutrients, particularly nitrogen, adding to the cost of production and finally leading to environmental pollution (Chandrasekharaih et al., 2011). An attempt has been made in the present study to investigated the effects of a probiotic, which may have a positive influence...
on rumen fermentation in ruminants (Jouany et al., 1991), when animals are affected by different protein levels. This study used two nitrogen (N) levels to test potential interactions (probiotics × N supply) and especially to study the different protein levels with or without probiotics on nutrient intakes, blood metabolites, nitrogen (N) balance and growth performance of Kurdi male lambs.

2. Material and methods

This study was conducted in Kurdi Animal Breeding Station in North Khorasan province, Iran.

2.2. Experimental design, animals, housing and diet

2.2.1. Experiment 1: pre-weaning phase

Twenty-four 10-d-old male fat-tailed Kurdi lambs with an average BW of 15.25 (SE ± 1.8 kg) were used in a 2×2 factorial arrangement to determine the effect of different protein levels with or without probiotics (Protexin®) in pre-weaning phase on growth performance, blood metabolites, rumen fermentation parameter and weaning stress. Single born lambs from third parity ewes were selected and were randomly divided into four groups; to be fed with 16% and 18% CP were supplemented with either 0 or 2 g/day of probiotics. The treatments were T1) 16% CP without probiotics; T2) 16% CP with probiotics; T3) 18% CP without probiotics; T4) 18% CP with probiotics. Lambs were housed in individual pens (2.5m×5m) and fresh drinking water was available at all times. The lambs fed twice daily were fed at 06:00 am and 16:00 pm. This experiment lasted 67 days, including 15 days feed adjustment period and fed their respective rations until weaning time (45 days of experiment) and 7 day after weaning. The ingredient and chemical composition of the diets used in first and second experiment is shown in Tables 1. Feed conversion ratio (FCR) was calculated by dividing the feed intake (g/day) by units of
weight gain (g/day) and average daily gain (ADG), daily feed intake (DFI) and feed efficiency (FE) were calculated.

2.2.2. Experiment 2: post-weaning phase

The experimental procedure was essentially the same as that for experiment 1 with minor exceptions. There were twenty-four male fat-tailed Kurdi lambs of 78-d-old, six per treatment, with an average weight of 29.38 (SE±3.1 kg). Experimental treatments consisted of a 2×2 factorial arrangement with two levels of protein with or without probiotics (Table 1). The lambs were randomly divided into four groups, to be fed with 14.5% and 16.5% crude protein were supplemented with either 0 or 2 g/day of probiotics. The treatments were T1) 14.5% CP without probiotics; T2) 14.5% CP with probiotics; T3) 16.5% CP without probiotics; T4) 16.5% CP with probiotics. This trial lasted for 60 days, including the first 15 days of adaptation. Lambs had ad libitum access to the diets, which were fed twice a day, at 06:00 am and 16:00 pm in equal portions of daily allowance with free access to water. Weight of each lamb was registered at the beginning of adaptation and feeding periods, and every 14 days throughout the feeding trial before removing the refusal and supplying fresh feed. Refusals were removed for calculation of voluntary dry matter intake. Samples from lambs within each treatment group were composited and stored for chemical analyses.

Table 1: Ingredient composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
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<tr>
<td>Lucerne hay</td>
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<td>20.00</td>
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<td>20.00</td>
<td>30.00</td>
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<td>T3</td>
<td>T4</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
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</tr>
<tr>
<td>Soybean meal</td>
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<td>11.20</td>
<td>17.20</td>
<td>17.20</td>
<td>4.90</td>
<td>4.90</td>
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<tr>
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<td>0.00</td>
<td>0.00</td>
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<td>0.35</td>
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<tr>
<td>Mineral and Vitamin Mix</td>
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<td>0.35</td>
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<td>Protexin®</td>
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<td>0.005</td>
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</table>

**Chemical composition**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>ME (Mcal/Kg DM)</th>
<th>Crude protein (%)</th>
<th>Neutral detergent fibre (%)</th>
<th>Acid detergent fibre (%)</th>
<th>Ash (%)</th>
<th>Calcium (%)</th>
<th>Phosphorus (%)</th>
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<tbody>
<tr>
<td>Soybean meal</td>
<td>2.81</td>
<td>16.00</td>
<td>27.40</td>
<td>51.00</td>
<td>5.71</td>
<td>0.64</td>
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</tr>
<tr>
<td>Calcium Carbonate</td>
<td>2.81</td>
<td>16.00</td>
<td>27.40</td>
<td>51.00</td>
<td>5.71</td>
<td>0.64</td>
<td>0.35</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>2.81</td>
<td>16.00</td>
<td>27.40</td>
<td>51.00</td>
<td>5.71</td>
<td>0.64</td>
<td>0.35</td>
</tr>
<tr>
<td>Urea</td>
<td>2.81</td>
<td>16.00</td>
<td>27.40</td>
<td>51.00</td>
<td>5.71</td>
<td>0.64</td>
<td>0.35</td>
</tr>
<tr>
<td>Salt</td>
<td>2.81</td>
<td>16.00</td>
<td>27.40</td>
<td>51.00</td>
<td>5.71</td>
<td>0.64</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Experiment details**

a T1 = 16% CP without probiotics; T2 = 16% CP with probiotics; T3 = 18% CP without probiotics; T4 = 18% CP with probiotics.

b T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics.

c 2 g/day probiotics

2.3. Blood and Rumen sampling

In experiment 1, blood samples were collected from the jugular vein into evacuated tubes on days 13 and 41 of the collection period for plasma glucose, blood urea nitrogen (BUN), triglycerides, cholesterol and total protein and for cortisol at 24 h before weaning and 24, 48
h after weaning. In experiment 2, blood samples were taken from the jugular vein (10-mL into sterile tubes containing EDTA solution) at 4 h after morning feed at the end of 28 days and 56 days of feeding trial. Blood samples were centrifuged at 3000 rpm for 5 min, and serum separated and stored at -20 °C for later analysis. Plasma glucose was determined using a spectrophotometric technique (UV-160A; Shimadzu, Tokyo, Japan) and BUN was measured by an automated enzymatic procedure. Serum cholesterol, cortisol, triglycerides, albumin, total proteins were measured by using an AutoAnalyzer.

Rumen liquor samples were taken two times 3 hours after feeding using a stomach tube. Ruminal pH was measured immediately after collecting the rumen fluid using a digital pH meter (Digital pH meter, 691 Metrohm, Herisau, Switzerland). Rumen liquor samples strained through four layers of cheesecloth and stored frozen pending analysis. Total N concentration of ruminal fluid was determined by micro-Kjeldahl (AOAC, 1990) using a digestion apparatus using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany).

2.4. Chemical analysis

Daily feed intake was taken and Samples of feeds and residues were analysed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) intake as per methods described by AOAC (1990).

2.5. Statistical analysis

Data were analyzed by analysis of variance procedures appropriate for a completely randomized design using the mixed procedures of SAS 9.1 (SAS Inst. Inc., Cary, NC). The Model statement was:

\[ y_{ijk} = \mu + p_l + p_xj + p_l \times px_j + time_k + p_l \times px_j \times time_k + (w_{1-w}) + e_{ijk} \]
Where $y_{ijk} =$ dependent variable; $\mu =$ overall mean of the population; $p_i =$ protein levels, $p_x = $ level of probiotic either 0 or 2 g/day; $(p_l \times px)_{ij} =$ interaction between protein levels and probiotic level; $W =$ initial weight as a covariate; $W_i =$ mean of initial weight and $e_{ijk} =$ unexplained residual element assumed to be independent and normally distributed. The means were compared using the Least Significant Difference option of General Linear Model procedure. For all analyses, probability values <0.05 were considered significant.

3. RESULTS and DISCUSSION

3.1. Experiment 1: pre-weaning phase

3.1.1. Growth performance

The results for growth performance of lambs fed diets containing different levels of protein and probiotics are shown in Table 2. Starter intake in pre-weaning phase was affected (P<0.05) by different protein and probiotics level diets. Starter intake was highest (P<0.05) in lambs fed a diet containing 18% CP and probiotics than other treatments. However, No difference (P<0.05) was noticed in starter conversion rate and daily gain in lambs fed diets containing different protein levels and probiotics (Table 2). The results obtained in this study on starter intake are similar to those reported by Titi et al. (2008), who observed no effect from supplementation of yeast culture on dry matter intake and growth rate in the diets of lambs and kids. Similarly, Whitley et al. (2009) observed no effect from probiotics supplementation on growth performance of goats, except in one growth trial in which weight gain and feed conversion rate was higher in probiotics.

Table 2: Effect of different protein levels with or without probiotics on growth performance pre-weaning (EXP. 1)
<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>15.31</td>
<td>15.20</td>
<td>15.28</td>
</tr>
<tr>
<td>Final weight (kg)</td>
<td>27.47</td>
<td>28.81</td>
<td>32.83</td>
</tr>
<tr>
<td>Starter intake (g DM/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 days</td>
<td>223.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>275.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>280.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>16-30 days</td>
<td>469.10</td>
<td>483.30</td>
<td>447.90</td>
</tr>
<tr>
<td>31-45 days</td>
<td>761.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>739.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>728.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>482.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>497.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>487.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After weaning</td>
<td>1173.40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1250.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1137.30&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Starter conversion ratio</td>
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<tr>
<td>0-15 days</td>
<td>0.98</td>
<td>0.98</td>
<td>1.04</td>
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<tr>
<td>16-30 days</td>
<td>2.80</td>
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<td>1.62</td>
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<td>31-45 days</td>
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<td>Total</td>
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<td>1.68</td>
<td>1.67</td>
</tr>
<tr>
<td>After weaning</td>
<td>5.30</td>
<td>5.61</td>
<td>5.30</td>
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<tr>
<td>Daily gain (g)</td>
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<tr>
<td>0-15 days</td>
<td>240.30</td>
<td>282.40</td>
<td>295.80</td>
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<tr>
<td>16-30 days</td>
<td>261.60</td>
<td>282.80</td>
<td>275.90</td>
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<tr>
<td>31-45 days</td>
<td>320.00</td>
<td>332.70</td>
<td>332.50</td>
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<tr>
<td>Total</td>
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<tr>
<td>After weaning</td>
<td>231.30</td>
<td>243.90</td>
<td>239.40</td>
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</table>

T1 = 16% CP without probiotics; T2 = 16% CP with probiotics; T3 = 18% CP without probiotics; T4 = 18% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

3.1.2. Blood metabolites
The results for blood metabolites of lambs fed diets containing different levels of protein and probiotics are shown in Table 3. Blood urea nitrogen (BUN) concentration in pre-weaning phase was not significantly affected (P<0.05) by probiotics supplementation in lambs fed 18% CP. BUN concentrations was significantly (P<0.05) lower in lambs fed 16% CP without probiotics than the other treatments. In addition, probiotics supplementation had influence BUN concentrations just in lambs fed 16% CP (Table 3). Blood urea nitrogen concentration can be useful indicator for monitoring protein status in animals. Higher BUN by lambs fed 18% CP diet might have resulted from higher N intake and its digestibility.

No difference (P<0.05) was noticed in blood glucose, triglyceride and total protein concentration across all the protein and probiotics level diets (Table 3). Interaction of protein level and probiotics had no effect on blood glucose, triglyceride and total protein concentration (Table 3). The lack of effect on blood glucose concentration in response to probiotic supplementation the diet is consistent with results reported by Antunovic et al. (2005) who reported unaltered blood glucose levels in the diets of lambs containing probiotics. While, Abo El-Nor and Kholif (1998) reported higher blood glucose concentration in cows fed diets containing probiotics. Ding et al. (2008) also found no effect from probiotics suplilmenation on blood glucose concentration in lambs.

**Table 3**: Effect of different protein levels with or without probiotics on blood metabolites pre-weaning (EXP. 1)

<table>
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<th>Items (mg/dL)</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
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<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
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<tr>
<td>Blood urea nitrogen</td>
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<td>d 13</td>
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<td>11.16&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Blood Glucose</strong></td>
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<tr>
<td>d 13</td>
<td>60.00</td>
<td>61.33</td>
<td>64.33</td>
</tr>
<tr>
<td>d 41</td>
<td>58.00</td>
<td>58.66</td>
<td>59.00</td>
</tr>
<tr>
<td>Total</td>
<td>59.00</td>
<td>60.00</td>
<td>61.66</td>
</tr>
<tr>
<td><strong>Blood triglyceride</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>50.00</td>
<td>50.33</td>
<td>55.66</td>
</tr>
<tr>
<td>d 41</td>
<td>35.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>47.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>42.50</td>
<td>47.83</td>
<td>51.66</td>
</tr>
<tr>
<td><strong>Blood total protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>5.36</td>
<td>5.73</td>
<td>5.33</td>
</tr>
<tr>
<td>d 41</td>
<td>5.43</td>
<td>5.46</td>
<td>5.36</td>
</tr>
<tr>
<td>Total</td>
<td>5.40</td>
<td>5.60</td>
<td>5.35</td>
</tr>
<tr>
<td><strong>Blood cortisol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h before weaning</td>
<td>13.20</td>
<td>13.00</td>
<td>13.50</td>
</tr>
<tr>
<td>24 h after weaning</td>
<td>17.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>48 h after weaning</td>
<td>16.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

T1 = 16% CP without probiotics; T2 = 16% CP with probiotics; T3 = 18% CP without probiotics; T4 = 18% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

No difference (P<0.05) was noticed in blood cortisol concentration across all the protein level diets, but blood cortisol concentration was affected due to probiotics supplementation (Table 3). Cortisol concentrations also changed during the weaning period. Increased blood cortisol concentrations were observed at 24h after weaning and were maintained 48 h after weaning. In addition, probiotics supplementation was significantly (P<0.05) reduced blood
cortisol concentrations in lambs. Protein and probiotics level interaction was non-significant (P<0.05) for blood cortisol concentration. Cortisol, a hormone secreted by the adrenal glands is the hormone of stress (Bravo et al. 2001). Some studies reported that the blood cortisol level tended to increase after weaning in calves (Zavy et al. 1992) and in camels (Mohamad et al., 2006).

3.1.3. Rumen fermentation parameter

The results for rumen fermentation parameter of lambs fed diets containing different levels of protein and probiotics are shown in Table 4. Ruminal pH in pre-weaning phase was unaffected (P<0.05) across the protein and probiotics level diets and no interaction was observed between protein and probiotics level on ruminal pH (Table 4). Wagner et al. (1990) reported that ruminal pH was not affected by probiotics supplementation (Aspergillus zae) fed to dairy calves and non-lactating cow diets. Ruminal ammonia-N was significantly affected (P<0.05) by different treatments. Ruminal ammonia-N concentrations was significantly (P<0.05) lower in lambs fed 16% CP than lambs fed 18% CP. In addition, probiotics supplementation had no influence ruminal ammonia-N concentrations in lambs fed 18% CP whereas, probiotics supplementation increased ruminal ammonia-N concentrations in lambs fed 16% CP (Table 4).

Table 4: Effect of different protein levels with or without probiotics on rumen pH and ammonia-N pre-weaning (EXP. 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>6.16</td>
<td>6.21</td>
<td>6.25</td>
</tr>
<tr>
<td>d 41</td>
<td>6.06</td>
<td>5.98</td>
<td>6.11</td>
</tr>
<tr>
<td></td>
<td>6.11</td>
<td>6.09</td>
<td>6.18</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Ammonia-N, mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>5.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 41</td>
<td>7.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>6.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

T1 = 16% CP without probiotics; T2 = 16% CP with probiotics; T3 = 18% CP without probiotics; T4 = 18% CP with probiotics. SE = standard error. Factor A = Protein level, Factor B = Probiotic level and AB = Interaction of protein level with probiotic level. NS = Non Significant and *= Significant (P<0.05).

3.2. Experiment 2: post-weaning phase

3.2.1. Growth performance

The results for growth performance of lambs fed diets containing different levels of protein and probiotics are shown in Table 5. Dry matter intake, feed conversation rate and daily gain in post-weaning phase were not significant different (P<0.05) in lambs fed diets containing different protein levels and probiotics (Table 5). Lambs that fed a diet containing 16.5% CP and probiotics had a higher significant (P<0.05) feed intake at 15 days after the beginning of experiment. Addition of probiotics did not influence (P<0.05) feed intake, feed conversation rate and daily gain. No interaction was observed between protein level and probiotics level on dry matter intake, feed conversation rate and daily gain (Table 5). The results of the present study are similar by Dabiri and Thonney (2004), who observed no significant effect from protein level on daily dry matter intake, but lambs fed diets with 15 or 17% CP were more efficient than lambs fed a diet with 13% CP. The effect of probiotics on dry matter intake is consistent with results reported by Hernandez et al. (2009), who obtained no significant change in dry matter intake of lambs fed grass diets with probiotics supplementation. Haddad and Goussous (2005) also reported similar findings. In addition, Swinney-Floyd et al. (1999) reported that supplementation of *Propionibacterium* and
Lactobacillus spp. independently or in a combination did not influence dry matter intake in calves. In addition, Sarwar et al. (2011) reported that dry matter intake were not significantly influenced (P<0.05) by probiotics supplementation in lambs.

Table 5: Effect of different protein levels with or without probiotics on growth performance post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Diets1</th>
<th>SEM</th>
<th>Significance</th>
</tr>
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<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 days</td>
<td>1435.6</td>
<td>1509.1</td>
<td>1370.9</td>
</tr>
<tr>
<td>16-30 days</td>
<td>1675.4</td>
<td>1698.1</td>
<td>1583.8</td>
</tr>
<tr>
<td>31-45 days</td>
<td>1676.9</td>
<td>1735.8</td>
<td>1726.6</td>
</tr>
<tr>
<td>total</td>
<td>1578.8</td>
<td>1639.3</td>
<td>1561</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 days</td>
<td>4.34</td>
<td>4.58</td>
<td>4.56</td>
</tr>
<tr>
<td>16-30 days</td>
<td>5.39</td>
<td>5.08</td>
<td>4.90</td>
</tr>
<tr>
<td>31-45 days</td>
<td>5.84</td>
<td>7.42</td>
<td>5.73</td>
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<tr>
<td>total</td>
<td>5.19</td>
<td>5.69</td>
<td>5.04</td>
</tr>
<tr>
<td>Daily gain (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 days</td>
<td>341.80</td>
<td>350.00</td>
<td>315.00</td>
</tr>
<tr>
<td>16-30 days</td>
<td>315.10</td>
<td>337.40</td>
<td>328.00</td>
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<td>31-45 days</td>
<td>291.30</td>
<td>265.50</td>
<td>300.40</td>
</tr>
<tr>
<td>total</td>
<td>316.70</td>
<td>317.90</td>
<td>316.60</td>
</tr>
</tbody>
</table>
T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB = Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

3.2.2. Blood metabolites

The results for blood metabolites of lambs fed diets containing different levels of protein and probiotics are shown in Table 6. Blood urea nitrogen (BUN) in post-weaning phase was significantly affected (P<0.05) by level of protein (Table 6). BUN concentrations were significantly (P<0.05) higher in lambs fed 16.5% CP than those fed 14.5% CP (Table 6). In contrast, addition of probiotics had no effect on BUN in lambs. There was no statistically interaction between protein source and probiotics for BUN (Table 6). The results of the present study are in agreement with the findings of Antunovic et al. (2005), who reported unaltered BUN concentration in lambs fed diets containing probiotics. Results from other studies reported that no difference (P<0.05) was noticed in BUN concentration due to probiotic supplementation compared with control group (Masek et al., 2008; Bruno et al., 2009).

Table 6: Effect of different protein levels with or without probiotics on blood metabolites post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Items (mg/dL)</th>
<th>Diets1</th>
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<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
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<tr>
<td>Blood urea nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>19.75b</td>
<td>21.00ab</td>
<td>23.75ab</td>
</tr>
<tr>
<td>d 56</td>
<td>20.75bc</td>
<td>18.00c</td>
<td>26.25a</td>
</tr>
<tr>
<td>Total</td>
<td>20.25ab</td>
<td>19.50b</td>
<td>25.00a</td>
</tr>
<tr>
<td></td>
<td>d 28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>d 56</td>
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<td>Total</td>
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<td></td>
</tr>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>63.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>61.75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>57.25&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>d 56</td>
<td>48.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.75&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Total</td>
<td>55.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>57.00&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood triglyceride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>17.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 56</td>
<td>18.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.75&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Total</td>
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<td>21.00&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>Blood cholesterol</td>
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<td></td>
</tr>
<tr>
<td>d 28</td>
<td>49.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 56</td>
<td>60.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.00&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>54.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.00&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>5.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 56</td>
<td>5.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.56&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Total</td>
<td>5.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

No difference (P<0.05) was noticed in blood glucose concentration across all the protein level diets (Table 6). In contrast to the results of pre-weaning phase, higher blood glucose concentration was observed on post-weaning phase in lambs fed probiotics diet (Table 6). The results of the second experiment are in agreement with the finding of Abo El-Nor and Kholif (1998) who reported that higher blood glucose concentration in cows fed diets containing probiotics. In lambs, the main source of glucose is from gluconeogenesis as it is
responsible for the satisfaction of 75% of the total glucose needs in ruminants (Donkin and Hammon, 2005). Higher blood glucose concentration observed in lambs fed diets supplemented with probiotics might be attributed to more nutrient digestibility resulting in increased precursor availability for gluconeogenesis.

The result indicated that there was significantly (P<0.05) effect of protein and probiotics level on blood triglyceride concentration (Table 6). Blood triglycerides was significantly (P<0.05) higher in lambs fed 16.5% CP than those fed 14.5% CP. The interaction between protein level and probiotics was non-significant (P<0.05) for blood triglyceride (Table 6). Blood total protein and cholesterol were significantly (P<0.05) affected by different treatments. Blood total protein were significantly (P<0.05) lower in lambs fed probiotics than in lambs fed diet without probiotics in 14.5% CP. There was a significant (P<0.05) interaction between protein and probiotics level for blood cholesterol and total protein in different diets (Table 6).

3.2.3. Rumen fermentation parameter

The results for rumen fermentation parameter of lambs fed diets containing different levels of protein and probiotics are shown in Table 7. Rumen pH in post-weaning phase was significantly (P<0.05) affected across the protein and probiotics level diets and no interaction was observed between protein and probiotics level for ruminal pH (Table 7).

Ruminal pH was significantly (P<0.05) lower in lambs fed 14.5% CP without probiotics than the other treatments. Ruminal pH is an important indicator of the rumen microbial ecosystem. The results of the present study showed that these probiotics are more effective to stabilize rumen pH in lambs fed 14.5% CP compared to lambs fed 16.5% CP. Probiotics can stabilize of ruminal pH by increasing the use of lactic acid by some ruminal bacteria (Nocek,
Following a study by Chiquette et al. (2008) it has been found that by administering a probiotic consisting of Prevotella bryantii in dairy cows results in normalization of ruminal pH and then reinstalling the normal digestion. Williams et al. (1989) reported that an increase in ruminal pH in steers fed diet supplemented by yeast culture (Saccharomyces cerevisiae).

In this study, ruminal ammonia-N concentrations were significantly (P<0.05) affected by different treatments in post weaning phase (Table 7). Ruminal ammonia-N concentrations was significantly (P<0.05) higher in lambs fed 16.5% CP than those fed 14.5% CP. No significant interaction was observed between protein and probiotics level in different diets (Table 7). Some studies reported that increasing protein concentration or degradation of diet usually resulted in increased ruminal NH3-N concentration (Klevesahl et al., 2003; Arroquy et al., 2004). Newbold et al. (1996) also found that NH3 concentrations increased when sheep were fed S. cerevisiae. However, Dawson et al. (1990) reported that the addition of S. cerevisiae to a fescue hay based diet fed to steers had no effect on NH3 concentrations.

**Table 7:** Effect of different protein levels with or without probiotics on rumen pH and ammonia-N post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>5.81</td>
<td>6.00</td>
<td>6.12</td>
</tr>
<tr>
<td>d 56</td>
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<td>total</td>
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<td>6.20</td>
</tr>
<tr>
<td><strong>Ammonia-N, mM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

### Conclusion

The findings of the present study imply that pre-weaned lambs fed diet with 18% CP with probiotics performed better starter intake than those fed diet with 16% CP with probiotics. Furthermore, probiotics supplementation did not significantly affected the growth performance of growing lambs in pre and pro weaning phase. Blood urea nitrogen and rumen ammonia-N concentration were higher in lambs fed diets with 18% CP than 16% CP in pre weaning phase. Blood cortisol concentrations were changed during the weaning period and probiotics supplementation was significantly reduced blood cortisol concentrations in lambs.

### References


Titi, H., Dmour, R., Abdullah, A., 2008. Growth performance and carcass characteristics of Awassi lambs and Shami goat kids fed yeast culture in their finishing diet. Animal Feed Science and Technology 142, 33-43.


Table 1: Ingredient composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Experiment 1 a</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2 b</th>
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<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
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<tr>
<td>Lucerne hay</td>
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<td>Barely</td>
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<td>18.9</td>
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<td>Calcium Carbonate</td>
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<td>0.35</td>
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</tr>
<tr>
<td>Magnesium Oxide</td>
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<td>0.00</td>
<td>0.00</td>
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<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td></td>
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</tr>
<tr>
<td>Mineral and Vitamin Mix</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
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<td></td>
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<tr>
<td>Urea</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.35</td>
<td>0.35</td>
<td>0.49</td>
<td>0.49</td>
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<tr>
<td>Salt</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
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<td>0.35</td>
<td>0.35</td>
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<tr>
<td>Sodium Bicarbonate</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.70</td>
<td>0.70</td>
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<tr>
<td>c Proteix®</td>
<td>0.00</td>
<td>0.005</td>
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Chemical composition

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<tr>
<th>Nutrient</th>
<th>Experiment 1 a</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2 b</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ME (Mcal/Kg DM)</td>
<td>2.81</td>
<td>2.81</td>
<td>2.81</td>
<td>2.81</td>
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<td>2.69</td>
<td>2.69</td>
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<td>Crude protein (%)</td>
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<td>16.00</td>
<td>18.00</td>
<td>18.00</td>
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<td>14.50</td>
<td>16.50</td>
<td>16.50</td>
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</tr>
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<td>Neutral detergent fibre (%)</td>
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<td>27.40</td>
<td>27.40</td>
<td>27.40</td>
<td>26.30</td>
<td>26.30</td>
<td>26.20</td>
<td>26.20</td>
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<tr>
<td>Acid detergent fibre (%)</td>
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<td>51.00</td>
<td>51.20</td>
<td>51.20</td>
<td>46.00</td>
<td>46.00</td>
<td>45.20</td>
<td>45.20</td>
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<td></td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.71</td>
<td>5.71</td>
<td>6.01</td>
<td>6.01</td>
<td>6.30</td>
<td>6.30</td>
<td>6.50</td>
<td>6.50</td>
<td></td>
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<tr>
<td>Calcium (%)</td>
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<td>0.64</td>
<td>0.66</td>
<td>0.66</td>
<td>0.50</td>
<td>0.50</td>
<td>0.51</td>
<td>0.51</td>
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</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.37</td>
<td>0.37</td>
<td>0.31</td>
<td>0.31</td>
<td>0.32</td>
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</table>

Table 2: Effect of different protein levels with or without probiotics on growth performance pre-weaning (EXP. 1)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>SEM A</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (kg)</td>
<td>15.31</td>
<td>15.20</td>
<td>15.28</td>
<td>15.04</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td>Final weight (kg)</td>
<td>27.47</td>
<td>28.81</td>
<td>32.83</td>
<td>31.86</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>Starter intake (g DM/day)</td>
<td>223.90</td>
<td>275.20</td>
<td>280.20</td>
<td>399.50</td>
<td>14.48</td>
<td>*</td>
</tr>
<tr>
<td>0-15 days</td>
<td>469.10</td>
<td>483.30</td>
<td>447.90</td>
<td>543.00</td>
<td>37.85</td>
<td>NS</td>
</tr>
<tr>
<td>16-30 days</td>
<td>761.60</td>
<td>739.60</td>
<td>728.60</td>
<td>830.20</td>
<td>25.27</td>
<td>NS</td>
</tr>
<tr>
<td>31-45 days</td>
<td>482.10</td>
<td>497.90</td>
<td>487.00</td>
<td>593.70</td>
<td>19.42</td>
<td>*</td>
</tr>
<tr>
<td>Total</td>
<td>1173.40</td>
<td>1250.60</td>
<td>1137.30</td>
<td>1267.40</td>
<td>24.84</td>
<td>NS</td>
</tr>
<tr>
<td>After weaning</td>
<td>0.98</td>
<td>0.98</td>
<td>1.04</td>
<td>1.35</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Starter conversion ratio</td>
<td>2.80</td>
<td>1.78</td>
<td>1.62</td>
<td>2.12</td>
<td>0.67</td>
<td>NS</td>
</tr>
<tr>
<td>0-15 days</td>
<td>2.52</td>
<td>2.27</td>
<td>2.30</td>
<td>2.34</td>
<td>0.21</td>
<td>NS</td>
</tr>
<tr>
<td>16-30 days</td>
<td>2.08</td>
<td>1.68</td>
<td>1.67</td>
<td>1.94</td>
<td>0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>0.53</td>
<td>5.61</td>
<td>5.30</td>
<td>4.94</td>
<td>0.59</td>
<td>NS</td>
</tr>
<tr>
<td>Daily gain (g)</td>
<td>240.30</td>
<td>282.40</td>
<td>295.80</td>
<td>296.80</td>
<td>25.05</td>
<td>NS</td>
</tr>
<tr>
<td>0-15 days</td>
<td>261.60</td>
<td>282.80</td>
<td>275.90</td>
<td>264.10</td>
<td>29.89</td>
<td>NS</td>
</tr>
<tr>
<td>16-30 days</td>
<td>320.00</td>
<td>332.70</td>
<td>332.50</td>
<td>357.50</td>
<td>29.24</td>
<td>NS</td>
</tr>
<tr>
<td>31-45 days</td>
<td>269.70</td>
<td>300.50</td>
<td>301.10</td>
<td>309.30</td>
<td>20.07</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>231.30</td>
<td>243.90</td>
<td>239.40</td>
<td>256.50</td>
<td>29.60</td>
<td>NS</td>
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</table>
Table 3: Effect of different protein levels with or without probiotics on blood metabolites pre-weaning (EXP. 1).

<table>
<thead>
<tr>
<th>Items (mg/dL)</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>10.00</td>
<td>12.08a</td>
<td>13.33a</td>
</tr>
<tr>
<td>d 41</td>
<td>12.33b</td>
<td>16.33a</td>
<td>17.66a</td>
</tr>
<tr>
<td>Total</td>
<td>11.16b</td>
<td>14.20a</td>
<td>15.50a</td>
</tr>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>60.00</td>
<td>61.33b</td>
<td>64.33b</td>
</tr>
<tr>
<td>d 41</td>
<td>58.00</td>
<td>58.66b</td>
<td>59.00b</td>
</tr>
<tr>
<td>Total</td>
<td>59.00</td>
<td>60.00b</td>
<td>61.66b</td>
</tr>
<tr>
<td>Blood triglyceride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>50.00</td>
<td>50.33b</td>
<td>55.66b</td>
</tr>
<tr>
<td>d 41</td>
<td>35.00b</td>
<td>45.33ab</td>
<td>47.66a</td>
</tr>
<tr>
<td>Total</td>
<td>42.50</td>
<td>47.83b</td>
<td>51.66b</td>
</tr>
<tr>
<td>Blood total protein</td>
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<tr>
<td>d 13</td>
<td>5.36</td>
<td>5.73b</td>
<td>5.33</td>
</tr>
<tr>
<td>d 41</td>
<td>5.43</td>
<td>5.46b</td>
<td>5.36</td>
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<tr>
<td>Total</td>
<td>5.40</td>
<td>5.60b</td>
<td>5.35</td>
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<tr>
<td>Blood cortisol*</td>
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<tr>
<td>24 h before weaning</td>
<td>13.20</td>
<td>13.00</td>
<td>13.50</td>
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<tr>
<td>24 h after weaning</td>
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<td>16.70b</td>
<td>17.13a</td>
</tr>
<tr>
<td>48 h after weaning</td>
<td>16.90a</td>
<td>16.06b</td>
<td>16.57ab</td>
</tr>
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</table>

Table 4: Effect of different protein levels with or without probiotics on rumen pH and ammonia-N pre-weaning (EXP. 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>6.16</td>
<td>6.21</td>
<td>6.25</td>
</tr>
<tr>
<td>d 41</td>
<td>6.06</td>
<td>5.98</td>
<td>6.11</td>
</tr>
<tr>
<td>Total</td>
<td>6.11</td>
<td>6.09</td>
<td>6.18</td>
</tr>
<tr>
<td>Ammonia-N, mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 13</td>
<td>5.02c</td>
<td>6.97b</td>
<td>6.72b</td>
</tr>
<tr>
<td>d 41</td>
<td>7.66b</td>
<td>9.50a</td>
<td>9.10ab</td>
</tr>
<tr>
<td>Total</td>
<td>6.34b</td>
<td>8.23a</td>
<td>7.91ab</td>
</tr>
</tbody>
</table>

T1 = 16% CP without probiotics; T2 = 16% CP with probiotics; T3 = 18% CP without probiotics; T4 = 18% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).
Table 5: Effect of different protein levels with or without probiotics on growth performance post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Diets&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

| Feed conversion ratio      |    |    |    |    |                  |
|                           |    |    |    |    |                  |
| 0-15 days                 |    |    |    |    |                  |
| T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).

Table 6: Effect of different protein levels with or without probiotics on blood metabolites post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Items (mg/dL)</th>
<th>Diets&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).
Table 7: Effect of different protein levels with or without probiotics on rumen pH and ammonia-N post-weaning (EXP. 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets¹</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>5.81b</td>
<td>6.00ab</td>
<td>6.12a</td>
</tr>
<tr>
<td>d 56</td>
<td>6.00b</td>
<td>6.16a</td>
<td>6.29a</td>
</tr>
<tr>
<td>total</td>
<td>5.90b</td>
<td>6.08a</td>
<td>6.20a</td>
</tr>
<tr>
<td>Ammonia-N, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 28</td>
<td>10.81b</td>
<td>11.00ab</td>
<td>11.12a</td>
</tr>
<tr>
<td>d 56</td>
<td>11.00b</td>
<td>11.16a</td>
<td>11.29a</td>
</tr>
<tr>
<td>total</td>
<td>10.90b</td>
<td>11.08b</td>
<td>11.20a</td>
</tr>
</tbody>
</table>

T1 = 14.5% CP without probiotics; T2 = 14.5% CP with probiotics; T3 = 16.5% CP without probiotics; T4 = 16.5% CP with probiotics. SE = standard error. Factor A= Protein level, Factor B= Probiotic level and AB= Interaction of protein level with probiotic level. NS= Non Significant and *= Significant (P<0.05).