Effect of Yeast Culture and its Combination with Direct-Fed Microbials on Growth Performance and Rumen Fermentation of Weaned Lambs

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Abstract

Thirty-two lambs weighing 22.20 (± 0.75) kg were randomly assigned to one of four treatments: No additive (control), yeast culture (YEC), YEC plus B. licheniformis (YBL) or YEC plus C. butyricum (YCB). Diet consisted of grass hay ad libitum and concentrate (300 g/head/day). Average daily gain was 102, 114, 90, and 89 g/d in control, YEC, YBL, and YCB, respectively, with no significant difference (P>0.05) among treatments, but the carcass weight of YEC lambs was significantly higher (P<0.05) than that of other treatments. Total volatile fatty acids and acetate to propionate ratio were uninfluenced, although the butyric acid concentration was higher (P<0.05) in the rumen fluid of YCB lambs compared to YEC lambs and slightly higher (P>0.05) than in controls and YBL lambs. Solid-associated fungi population relative to total rumen bacteria 16S ribosomal DNA was significantly lower (P<0.05) in YBL lambs (3.55) compared to YCB (23.12). There was little difference in blood glucose and plasma urea-N concentrations among the treatments. Blood concentrations of creatinine and globulin were significantly higher (P<0.05) in YBL and YCB lambs compared to the control and YEC-fed animals. Results indicated that yeast culture improved growth performance with little advantage expected from combining yeast culture with either B. licheniformis or C. butyricum. More research using adjusted diet formulations and doses of B. licheniformis or C. butyricum in combination with yeast culture is recommended.

Keywords: Direct fed microbials, ruminant nutrition, sheep

Introduction

Ruminant productivity in many parts of the world is restricted by nutritional deficits in terms of amount and quality of available forages. However, productivity can be improved by improving the digestion and utilization of available nutrients. The nutrition of ruminants is uniquely dominated by microbial fermentation in the gut, therefore, technologies such as direct-fed microbials (DFMs) have been employed to manipulate the rumen environment into improving nutrient digestibility (Bio-Vet 2019). The beneficial effects of yeast culture have already been demonstrated in various studies around the world (Khan *et al.* 2016).

In China, several studies have been conducted to determine the effect of DFMs, such as *Bacillus licheniformis* and *Clostridium butyricum*, on porcine performance. Little is known about the effects these DFMs may have in ruminants. In a preliminary study (Doto and Liu, 2011), it was suggested that *B. licheniformis* and *C. butyricum* alone may not directly affect fiber digestibility, but may indirectly stimulate cellulolytic microbes in the rumen through cross-feeding mechanisms. It is hypothesized that fiber digestibility may be further improved by combining *B. licheniformis* or *C. butyricum* with yeast culture through synergistic effects.

Studies have shown that beneficial effects of DFMs are more pronounced in stressed animals with little advantage being observed in healthy mature animals (Buntyn *et al.* 2016, Hutjens 2007). Weaning is a critical transition in the life

of a ruminant, the transition from milk to solid food may cause a lag in growth (Di Francia *et al.,* 2008). The objective of this study was to evaluate the effect of *B. licheniformis* and *C. butyricum* combinations with yeast culture on the growth performance of weaned lambs.

Materials and Methods

DFMs and yeast culture

B. licheniformis and *C. butyricum* (Zhejiang Future, Hangzhou, China) were in powder form consisting of the bacteria and their respective carrier fermentation media. Colony forming units (cfu) of *B. licheniformis* and *C. butyricum* were 1.0×10^{11} and 3.8×10^{8} per g, respectively. Yeast culture was also in powder form (Diamond V Mills Inc., Iowa, USA), and included yeast (Saccharomyces cerevisiae) and the media on which the yeast grew.

Animals, feed and experimental design

Thirty-two male lambs of Hu sheep, approximately 90 days of age and weighing 22.20 ± 0.75 kg were blocked according to live-weight and randomly assigned to four groups of eight animals each. The four treatments were: (1) no additive (control); (2) yeast culture at 15 g/d (YEC); (3) YEC plus 2.3 g/d *B. licheniformis* (YBL); and (4) YEC plus 2.3 g/d *C. butyricum* (YCB). All animals were maintained on Chinese wildrye (Leymus chinensis) grass hay ad libitum and 300 g/head/d of a concentrate mixture containing corn (650 g/kg), wheat bran (150 g/kg), bean residue (160 g/kg), $Ca(H_2PO_4)_2$ (30 g/kg) and NaCl (10 g/kg). Feeding was at 0800h and 1600h and animals had free access to drinking water. Yeast culture and DFMs for the respective treatment groups were mixed in the concentrate before feeding. The feeding trial lasted 75 d with 15 d for adaptation.

Parameters measured

Growth performance and carcass measurements

Lambs were weighed before the morning feeding at the beginning of the experiment and every 15 d. At the end of the growth trial, four lambs from each treatment were slaughtered to determine carcass weight, dressing percentage, rib-eye area and GR value. GR value is the depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the 12th rib 110mm from the midline.

Rumen fermentation characteristics and microbial populations

Samples of rumen contents were taken immediately after slaughter to be analyzed for end-point pH, volatile fatty acids (VFA), ammonia-nitrogen, ciliate protozoa numbers and populations of solid- and liquid-associated microbes. Samples were strained through four layers of gauze for separation of solid from liquid and divided into aliquots for the various analytical procedures. Rumen pH was determined using a pH meter, ciliate protozoa were counted using a hemacytometer under microscope, ammonia-nitrogen and VFA were determined using the methods described by Hu *et al.* (2005). Solid- and liquid- associated microbes were determined by isolation of total DNA from liquid- and solid-associated microbes using the bead-beating method as described by Zoetendal *et al.* (1998) with minor modifications, and quantification of total bacteria, fungi, *R. flavefaciens* and *F. succinogenes* by species-specific real time qPCR as described by Denman and McSweeney (2006).

Growth-related blood biochemical metabolites

Samples of serum from blood drained via the jugular vein at slaughter were analyzed for glucose, urea-nitrogen, creatinine, total protein, albumin and globulin concentrations using biochemical reagent kits from Sigma.

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS (1999). One-way analysis of variance was performed and comparisons of means among treatments were made using Duncan's multiple range test. Significant differences were accepted if $P \leq 0.05$.

Results

Growth performance and carcass traits

No significant effect of treatment on daily gain, dressing percentage, rib-eye area and GR value was observed ($P>0.05$, Table 1). Final carcass

Table 1: Production performance and carcass quality in weaned lambs fed diets with or without direct-fed microbials

Items	Treatment[1]				SEM	P
	Control	YEC	YBL	YCB		
Growth performance						
Initial weight (kg)	22.1	22.5	21.9	22.3	0.75	NS
Final weight (kg)	29.7^{ab}	31.0^{a}	28.7^{b}	29.0^{ab}	0.79	*
Daily gain (g/day)	101.6	113.5	90.1	89.4	9.15	NS
Carcass quality						
Carcass weight (kg)	13.0^{b}	14.2^{a}	13.5^{ab}	13.2^{ab}	0.32	*
Dressing percent (%)	44.7	45.6	46.5	45.9	0.88	NS
Rib-eye area (cm^2)	12.0	12.3	13.2	11.8	1.00	NS
GR value[2]	0.95	1.10	0.93	1.00	0.106	NS

[1] Control = no additive; YEC = yeast culture at 15 g/d; YBL = YEC and B. licheniformis at 2.3 g/d, respectively; and YCB = YEC and C. butyricum at 2.3 g/d, respectively.
[2] GR value: The depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the 12th rib 110mm from the midline.
*P<0.05; NS=not significant
a,b Within a row, means without a common superscript letter differ (P<0.05).

weight was significantly higher (P<0.05) in YEC animals than controls.

Rumen fermentation parameters and microbial populations

Treatment did not have a significant influence (P>0.05) on rumen pH and ammonia-nitrogen (Table 2). Total VFA and acetate to propionate ratio were not affected, but butyric acid concentration was significantly higher (P<0.05)

Table 2: Rumen pH, ammonia-nitrogen, volatile fatty acid, and ciliate protozoa variables for weaned lambs fed diets with or without direct-fed microbials

Items[1]	Treatment[2]				SEM	P
	Control	YEC	YBL	YCB		
Rumen pH	6.86	6.69	6.82	6.97	0.178	NS
Ammonia-N (mg/dL)	9.86	8.78	12.57	9.03	1.428	NS
Total VFA (mmol/L)	32.4	34.4	34.7	34.7	7.52	NS
Molar proportion (%)						
Acetate	82.2	79.6	80.8	77.3	19.85	NS
Propionate	15.0	18.3	16.2	17.3	2.45	NS
Butyrate	2.71^{ab}	2.15^{b}	3.08^{ab}	4.76^{a}	0.673	*
Ac / Pr ratio	5.24	4.26	4.85	4.55	0.739	NS
Ciliate protozoa (x10^5/mL)						
Holotrichs	0.19	0.00	0.50	0.13	0.191	NS
Oligotrichs	1.00	0.69	1.00	0.94	0.271	NS
Total	1.19	0.69	1.50	1.06	0.256	NS

[1]VFA=Volatile fatty acid; Ac / Pr ratio=Ratio of acetate to propionate
[2] Control = no additive; YEC = yeast culture at 15 g/d; YBL = YEC and B. licheniformis at 2.3 g/d, respectively; and YCB = YEC and C. butyricum at 2.3 g/d, respectively.
*P<0.05; NS=not significant
a,b Within a row, means without a common superscript letter differ (P<0.05).

in the rumen fluid of lambs receiving YCB compared to YEC lambs. The ciliate protozoa population in rumen fluid was not influenced (P>0.05) by treatment. There was no significant effect (P>0.05) of addition of yeast culture and its combination with DFMs on solid- and liquid-associated populations of R. flavefaciens, *F. succinogenes* and Fungi (Table 3). However, the solid-associated fungi population size was significantly lower (P<0.05) in YBL lambs compared to YCB animals.

lambs treated with YBL. Significantly higher (P<0.05) albumin was observed in the serum of lambs treated with YCB. Lambs receiving YBL or YCB had significantly higher (P<0.05) serum creatinine levels.

Discussion

Differences in daily gain between treatments were not statistically significant (P > 0.05) due to variability in live-weight measurements caused by differences in the weight of contents of the

Table 3: Effects of addition of yeast culture and direct-fed microbials on the population of solid- and liquid-associated microbes (% of total bacterial 16 S rDNA)

Items	Treatment[1]				SEM	P
	Control	YEC	YBL	YCB		
Solid-associated						
Fungi	14.018[ab]	9.75[ab]	3.55[b]	23.12[a]	5.599	*
R. flavefaciens	1.54	2.11	4.94	4.13	2.138	NS
F. succinogenes	3.19	4.74	9.97	5.16	3.570	NS
Liquid-associated						
Fungi	0.25	1.10	0.09	0.07	0.562	NS
R. flavefaciens	0.05	0.94	0.01	0.16	0.372	NS
F. succinogenes	19.53	9.68	2.16	3.56	9.700	NS

[1] Control = no additive; YEC = yeast culture at 15 g/d; YBL = YEC and *B. licheniformis* at 2.3 g/d, respectively; and YCB = YEC and *C. butyricum* at 2.3 g/d, respectively.
*P<0.05; NS=not significant
a,b Within a row, means without a common superscript letter differ (P<0.05).

Blood biochemical parameters

There was no significant influence of treatment (P>0.05) on blood glucose and urea-nitrogen (Table 4). Total protein and globulin in blood serum was significantly higher (P<0.05) in

digestive tracts between animals. However, the significantly higher (P<0.05) carcass weight observed in YEC lambs compared to controls was probably due to yeast culture increasing the efficiency of utilization of energy for fattening

Table 4: Serum biochemical parameters for weaned lambs fed diets with or without direct-fed microbials

Items	Treatment[1]				SEM	P
	Control	YEC	YBL	YCB		
Glucose (mmol/L)	4.60	4.67	5.36	5.89	0.432	NS
Urea nitrogen (mmol/L)	3.72	3.60	3.74	3.65	0.298	NS
Creatinine (µmol/L)	77.3[b]	79.6[b]	97.9[a]	92.1[ab]	3.55	**
Total protein (g/L)	57.1[b]	61.7[ab]	71.0[a]	66.5[ab]	1.99	**
Albumin (g/L)	24.8[b]	27.4[ab]	28.3[ab]	29.0[a]	1.04	*
Globulin (g/L)	32.3[b]	34.3[b]	42.7[a]	37.5[ab]	1.46	**

[1] Control = no additive; YEC = yeast culture at 15 g/d; YBL = YEC and B. licheniformis at 2.3 g/d, respectively; and YCB = YEC and C. butyricum at 2.3 g/d, respectively.
*P<0.05; ** P<0.01; NS=not significant
[a,b] Within a row, means without a common superscript letter differ (P<0.05).

via its ability to decrease the proportion of acetate relative to propionate in the rumen liquor (Plata *et al.,* 1994). Although acetate to propionate ratio was only slightly lower in YEC lambs compared to controls, it is possible that a combination of factors affected carcass weight in this study. For instance oligotrich protozoa numbers were slightly lower in YEC lambs compared to controls. These protozoa are predatory to rumen bacteria, therefore, if their population is decreased by treatment it can lead to improved bacterial protein yield and supply to the host animal and subsequently improve animal performance. Also solid-associated *R. flavefaciens* and *F. succinogenes* populations were slightly increased by treatment, suggesting that these particular species improved cellulolysis and nutrient availability in treated lambs slightly. Another factor is that cellulolytic microbes are very sensitive to rumen pH and require the pH level to stay between 6.2 and 6.8 in order to multiply rapidly (Ørskov, 1982; Leek, 1993). Although there was no significant difference in rumen pH between treatments, YEC lambs tended to have a more optimal rumen pH for cellulolysis compared to other treatments. Rumen ammonia-nitrogen was also slightly lower in YEC lambs compared to other treatments suggesting that slightly more ammonia-nitrogen was incorporated into microbial protein, which is biologically more valuable to the host ruminant when digested in the abomasum and duodenum. Lambs receiving YEC also benefitted slightly from higher rumen VFA than controls as VFAs contribute about 70% of a ruminant's total energy requirements (Banerjee, 1998).

The insignificant difference ($P > 0.05$) in carcass weight observed in YBL and YCB lambs compared to controls was unexpected according to the hypothesis; however, it may be the result of inadequate nutrient availability from the diet. Based on blood creatinine and glucose results, animals in YBL and YCB treatments appear to have suffered from the effects of increased glucose demand (Overton *et al.,* 1999). When ruminants face a sudden increased glucose demand, the liver increases its capacity to produce glucose from amino acids. Increased degradation of skeletal muscle protein seems to accompany this increased capacity for liver conversion of amino acids to glucose, which would supply amino acids to the liver for conversion into glucose (Overton *et al.,* 1999). This implies that more careful consideration was needed for diet formulation relative to protein and amino acid nutrition. Although amounts of absorbable protein in the blood were improved with treatment, efficiency of protein utilization was not improved as indicated by plasma urea-N and in YBL and YCB lambs it seems the rate of skeletal muscle degradation was greater than protein retention.

Conclusion

From the current study it was concluded that supplementation with yeast culture alone improved growth performance significantly. Economically, there was no added advantage of combining yeast culture with either *B. licheniformis* or *C. butyricum*. Further research on the combination of yeast culture with either *B. licheniformis* or *C. butyricum* using adjusted diet formulations and DFM dose levels is recommended.

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