



Research article

Effect of dietary inclusion of graded levels of distillers dried grains with solubles on the performance, blood profile and rumen microbiota of Najdi lambs

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ABSTRACT

An experiment was conducted in a completely randomized design to evaluate the effect of including graded levels (0, 20, 30, 40 and 50% of diet) of dried distiller's grains with solubles (DDGS) on growth performance, slaughter parameters, blood serum metabolites and rumen microbiota in weaned Najdi male lambs. Thirty-five lambs, initial body weight of 33.45 ± 0.75 kg, and approximately three-month old were used in a 94-day feeding experiment. Performance measurements were conducted biweekly and blood samples were collected monthly. Inclusion of DDGS in the diets of growing Najdi lambs at levels up to 50% did not affect body weight gain (BWG) compared with the lambs fed the control diet (CON, 0% DDGS). Lambs fed the 50% DDGS diet consumed less feed compared with lambs in other groups (98 vs 112.5 kg DM) but had no adverse effect on BWG. Rumen pH values at 0, 6, 12 and 18 h post feeding and concentrations of blood serum total proteins, glucose, triglycerides, urea-N or albumin were similar across treatments. Slaughter parameters including slaughter weight, hot and cold carcass weights and dressing % (hot and cold carcass) were not affected by the treatments. There was no difference in the weights of full compartmental stomach and intestines, liver, omental fat, Kidney Knob and Channel Fat (KKCF) and tail fat between DDGS treatments and CON. The study concluded that the inclusion of DDGS in the diets of growing Najdi lambs had no adverse effects on growth performance and slaughter parameters. Rumen microbiota was not affected, however, our data suggest significant interactions between DDGS and selected bacterial groups and DDGS driven rearrangement of *Prevotella* species.

1. Introduction

Dried distillers' grains with solubles (DDGS), a by-product of the newly emerged ethanol industry, are produced during the corn-to-ethanol production process. The global biofuels industry produces about 52 million metric tons (MT) of DDGS for use in animal feed. The United States alone produces about 38 million MT, which is comparable to the amounts of soybean meal produced in the US annually. Of this 38 million MT, approximately 66% is domestically consumed, while the remaining 34% is exported to 30 countries around the world (Shurson, 2019). The boom in the ethanol production sector led to higher availability of DDGS for livestock feed (Liu et al., 2011), and stimulated

interest by researchers to evaluate its value as a protein and energy source for the different species of animals. On the other hand, the struggle to control feed cost in livestock sector continues worldwide. The policy makers in Saudi Arabia have shifted from planting feed to importing animal feed and by-products due to water scarcity. Of these by-products, DDGS has received the most attention, and the import of this commodity together with corn gluten feed (CGF) has risen to 88,014 MT during the year 2017/2018. The importers of DDGS and CGF received import subsidies of \$99 and \$91 per MT, respectively (Mousa, 2019), this subsidy was, however, recently removed.

From the nutritional point-of-view, DDGS contains higher levels of phosphorus than corn; thus, adding DDGS to an animal's diet may negate

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or reduce the need for phosphorus supplements. Inclusion of DDGS at levels up to 183 g/kg feed DM improves rumen conditions by increasing rumen pH, and enhance fiber digestion in the rumen (Eun et al., 2009). This could result in major alterations in native rumen microbial community and lead to intestinal and health issues. The growing interest in the use of DDGS as an alternative animal feed has increased dramatically worldwide (Popp et al., 2016), nevertheless the effects on rumen microbiota are yet to be further investigated.

High-quality DDGS is a potentially useful feed ingredient, and the average chemical composition on 100% dry matter basis of 118 samples of DDGS, with coefficients of variations in parenthesis, was 30.2 (6.4%) crude protein (CP), 10.9 (7.8%) crude fat, 8.8 (8.7%) crude fibre, 5.8 (14.7%) ash, 16.2 (28.4%) ADF and 42.1 (14.3%) neutral detergent fibre NDF (Spiehs et al., 2002). In addition, the composition of DDGS collected at one plant over a five-year period was approximately 31.3% crude protein, 11.9% crude fat, 10.2% crude fibre and 4.6% ash (Belyea et al., 2004), 31.8% neutral detergent fibre (NDF) and 19.8% acid detergent fibre (ADF) (Wen et al., 2010).

Traditionally, barley and corn are the main sources of dietary energy for fattening ruminant animals, which causes a major reduction in fibrolytic bacteria and a rapid growth of amylolytic bacteria due to their readily fermented starch contents (Goad et al., 1998; Tajima et al., 2001). Consequently, their digestion lowers ruminal pH, feed intake and animal performance. DDGS contains similar or more energy than barley grain (NRC, 1985). The energy in DDGS is primarily derived from the digestible fibre and fat while in barley, most of the energy is in the form of starch (Avila-Stagno et al., 2013).

Experimental data suggest that inclusion of DDGS in the diet of finishing lambs at 23% does not have adverse effects on growth performance and carcass quality measures (Huls et al., 2006). Many studies reported no differences in the general performance and carcass characteristics in finishing lambs when corn was substituted with DDGS up to 60% (Schauer et al., 2008; Neville et al., 2010; Wood et al., 2011; Van Emon et al., 2012). Klopfenstein et al. (2008) reported that DDGS had an increased feeding value for cattle compared to corn when included at 10 and 20% of feedlot rations. However, a meta-analysis of the reported data suggested that as DDGS levels increase from 0 to 40% in the ration of feedlot steers, the marbling score decreased linearly with a concomitant linear increase in yield grade (Klopfenstein et al., 2008). Eun et al. (2009) reported no differences in the growth performance of steers fed DDGS diets containing 10.5–18.3% DDGS during growing and finishing periods. Similarly, DM and ADF digestibility were not affected, but DMI decreased with increasing DDGS. Further, DDGS feeding tended to increase NDF digestibility and ruminal pH and decrease volatile fatty acids (VFA) production in the rumen (Eun et al., 2009).

DDGS was also used in the diets of lactating Awassi ewes and their growing ewe lambs. Inclusion of DDGS increased milk yield and had no effect on body weight in the lactating ewes (Alpizar-Rodriguez et al., 2019). Digestibility of CP and NDF was higher for the DDGS diet. Curzayn-Leyva et al., 2019 investigated the impact of replacing barley and soybean meal with DDGS on the performance and carcass weight of Creole wool lambs. They concluded that the inclusion of DDGS at 15% improves hot and cold carcass weights, both were heavier in DDGS diets.

The objective of this experiment was to evaluate the effect of feeding different levels of DDGS (0, 20, 30, 40 and 50%) in the total mixed ration (TMR) on growth performance, slaughter parameters and some blood metabolites in growing Najdi lambs and the impact on rumen microbiota structure and diversity.

2. Materials and methods

Thirty-five growing, 3-month-old Najdi male lambs with the live body weight of 33.68 ± 0.71 kg were used in this experiment. Animals were individually housed (0.8×1.4 m) with a separate feeder and drinker and injected subcutaneously with 2 ml of enterotoxaemia vaccine. Lambs were randomly divided into five equal groups and fed total mixed ration

(TMR) containing 0% DDGS (control group, CON), 20% DDGS, 30% DDGS, 40% DDGS, and 50% DDGS. TMR was formulated to meet the growing lambs requirements (Table 1) according to NRC tables of requirements (NRC, 1985). Lambs were fed the assigned diets for a preliminary adaptation period of two weeks, followed by an experimental period for 94 days. For rumen pH measurements, 15 lambs fitted with permanent rumen cannula were randomly divided into five equal groups ($n = 3$) and each assigned to one of the experimental diets. Rumen pH was measured immediately after collection at 0, 6, 12, 18 h post-feeding.

This project was approved by the Departmental Board of Studies on Ethics, Methodology and Welfare, King Saud University, Kingdom of Saudi Arabia. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.1. Measurement

Lambs initial body weights (BW) were recorded, and subsequently, BWs were taken biweekly after overnight fasting. Feed intake, BW gains (BWG) and feed conversion efficiency (FCE) were calculated weekly (kg BWG/kg DM feed intake). Blood samples were collected via jugular vein at the beginning of the trial and subsequently at a monthly interval. Blood samples were centrifuged at 3000 rpm for 15 min and serum was separated and stored at -80°C (AOAC, 1990). Serum samples were analyzed for glucose, triglycerides, urea-N, albumin and total protein using commercial kits (Randox Laboratories Limited, United Kingdom). At the end of the experiment, 25 lambs were slaughtered to study slaughter parameters (Osman and Aldosari, 2006). Rumen fluid samples (200 ml) were collected postmortem for extraction of genomic DNA and analysis of rumen microbiota.

2.2. Statistical analyses

Animal trial data were analyzed as a complete randomized design (CRD) using Statgraphics Plus (1996, Manugistics, Rockville, MD). Comparisons between means were made using Fisher's least significant differences (LSD) procedures with probability (P) of <0.05 considered statistically significant.

2.3. DNA extraction and sequencing of 16S rRNA gene

Rumen fluid samples were collected post mortem and the DNA was extracted using the method of Yu and Morrison (2004) with modification as in Stanley et al. (2012). After confirming DNA quality with gels and nanodrop, DNA amplification was performed using primers selected to amplify the V3–V4 region of 16S rRNA genes: forward ACTCCTACGG-GAGGCAGCAG and reverse GGACTACHVGGGTWTCTAAT. Sequencing was performed on the Illumina MiSeq platform using 2×300 bp paired-end sequencing and the sequencing protocol proposed by Fadrosch et al. (2014). The data analysis was done in Qiime (Caporaso et al., 2010). Sequence joining parameters were 0% error in the overlapping region and the minimum accepted Phred quality of 20. Operational Taxonomic Units (OTUs) were picked using Uclust algorithm (Edgar, 2010) at 97% sequence identity and chimeric sequences were inspected with Pintail (Ashelford et al., 2005). Taxonomy was assigned using blast against the Green Genes database (DeSantis et al., 2006) and the data were visualised and analyzed using Calypso (Zakrzewski et al., 2016).

We sequenced seven rumen fluid samples from each treatment; 2 samples failed (low number of sequences), and 33 samples passed the required quality control. Two groups (CON and DG20) were represented with 6 and all remaining groups with seven sequenced samples per treatment. Distribution of sequence number per sample was inspected; the smallest sequenced sample was comprised of 11,144 sequences and largest of 25,983. There were no significant differences (ANOVA) between treatments in the number of sequences per sample (Figure 1A). We used OTU table filtered to remove OTUs with relative abundance lower than 0.01% and performed Hellinger transformation (Total Sum

Table 1. Dietary ingredients (%) and chemical composition of the experimental diets.

Dietary Ingredients	Levels of DDGS [†] (% fresh basis)				
	0% (CON)	20% (T1)	30% (T2)	40% (T3)	50% (T4)
Barley	60.8	49	41.4	32.8	32.8
DDGS	0	20	30	40	50
SBM	18	8	6	4	0
Wheat bran	4	5	5	6	4.7
Alfalfa hay	14	15	15	15	10
Urea	1	0.8	0.4	0	0
CaCO ₃	1.5	1.5	1.5	1.5	1.8
Salt	0.5	0.5	0.5	0.5	0.5
Min & Vit Premix	0.2	0.2	0.2	0.2	0.2
Chemical composition (%DM)					
Dry matter	91.9	92.3	92.7	93.1	93.6
Crude protein	22.97	22.88	21.93	22.81	22.39
Ether extract	1.50	3.56	3.33	4.32	5.19
Crude fibre	6.30	7.34	8.34	8.11	7.81
Ash	5.38	5.54	5.81	5.99	5.54
ME [‡] , MJ/kg DM	11.83	11.77	11.75	11.72	11.71

[†] DDGS = Dried Distillers Grains with Solubles, CON, control.

[‡] ME = Metabolisable energy, calculated and expressed as MJ per kg DM using NRC feed tables (NRC 1985).

Normalisation on square-root transformed data) (Legendre and Galagher, 2001). ANOVA was used to inspect the difference between the treatments. Multivariate analysis was done using Discriminant Analysis of Principal Components (DAPC) and also Adonis and Anosim, both on Weighted and Unweighted Unifrac distance and with 999 permutations. The difference in diversity was estimated using ANOVA on Richness, Evenness, Shannon and Inverse Simpson's index. We inspected both Pearson and Spearman correlations of microbial taxa against the concentration of DDGS using regression analysis in Calypso.

The fully annotated dataset for this study is available on the MG-RAST server (<http://metagenomics.anl.gov/>) under project ID mg1816341.

3. Results

3.1. Chemical composition of the experimental diets

The DDGS diets had similar chemical composition to the CON except for the ether extract fraction (crude fat), which was higher in the DDGS diets, reaching the highest level of 5.19% in 50% DDGS diet compared with the lowest level of 1.5% for the CON diet (Table 1).

3.2. Feed intake, rumen pH and growth performance

Feed intake (FI), body weight gain (BWG) and feed conversion efficiency (FCE) are presented in Table 2. Feed intakes, BWG and FCE were similar ($P > 0.05$) across treatments. Although insignificant, the total FI for the 94-day feeding period was 98 kg for lambs in the 50% DDGS diet compared with an average intake of 112.5 kg for the lambs fed CON and other DDGS diets. Rumen pH values were similar across treatments ($P = 0.189$) and sampling time ($P = 0.941$). pH values for the CON, 20% DDGS, 30% DDGS, 40% DDGS and 50% DDGS were 6.37, 6.14, 6.45, 6.18 and 5.89, respectively.

3.3. Blood serum metabolites

The concentrations of total proteins, glucose, triglycerides, urea-N and albumin in blood serum of the growing Najdi male lambs are reported in Table 3. Treatments had no influence on the concentrations of the measured blood serum parameters. Values for the monthly samples were averaged and presented as the concentrations for the overall period.

Serum concentrations of total proteins, glucose, triglyceride, urea-N and albumin averaged 10.18 ± 0.47 g/dL, 96.97 ± 8.28 mg/dL; 23.35 ± 1.4 mg/dL; 57.14 ± 3.27 mg/dL and 4.0 ± 0.11 mg/dL, respectively.

3.4. Slaughter parameters and carcass characteristics

Slaughter parameters are shown in Table 2. Inclusion of DDGS at different levels had no effect on slaughter weights, hot and carcass weights and dressing percentages. There was no effect of inclusion of DDGS on omental or KKCF in lambs. The full stomach weight in lambs fed the 50% DDGS diet in this present study was 1.36 kg heavier than other groups (6.53 compared with an average of 5.17 for the other groups).

3.5. DDGS induced changes in rumen microbiota structure

Bacteroidetes dominated rumen microbiota of Najdi lambs, and to a lesser extent, Firmicutes. Rumen microbiota was comprised of 12 phyla (Figure 1B). At the genus level, the dominance of *Prevotella* was prominent (Figure 1C). ANOVA on alpha diversity indicators showed that DDGS did not change Richness ($P = 0.29$), Evenness (or Dominance) ($P = 0.35$), Shannon ($P = 0.25$) or Inverse Simpson Index ($P = 0.30$).

Adonis multivariate analysis on Weighted ($P = 0.502$) and Unweighted Unifrac ($P = 0.513$) was showing no significant differences between the treatments, while Anosim analysis demonstrated that DDGS treatments did not influence the difference in variation of microbiota within nor between the treatments (Weighted Unifrac $P = 0.647$; Unweighted Unifrac $P = 0.523$) in either species number or abundance. When introducing such a unique, high fibre additive like DDGS, the possibility that it could make drastic changes in microbiota are of concern, as immense changes to a well-balanced system such as rumen would unlikely be of benefit. It is also important that (based on Anosim) high concentrations of DDGS did not introduce sheep to sheep variation within the treatment, but instead, that the response of these microbiotas to high DDGS content was reproducible and reliable.

We then used the Discriminant Analysis of Principal Components (DAPC) to look deeper into relationships between the groups. Discriminant Analysis of Principal Components is a multivariate method designed to identify and describe clusters of related individuals (Jombart et al., 2010). DAPC showed a clear distinction of the CON group from all DDGS supplemented groups at the OTU level (Figure 2); this was driven by the differences in the distribution of several *Prevotella* OTUs (Figure 2). This

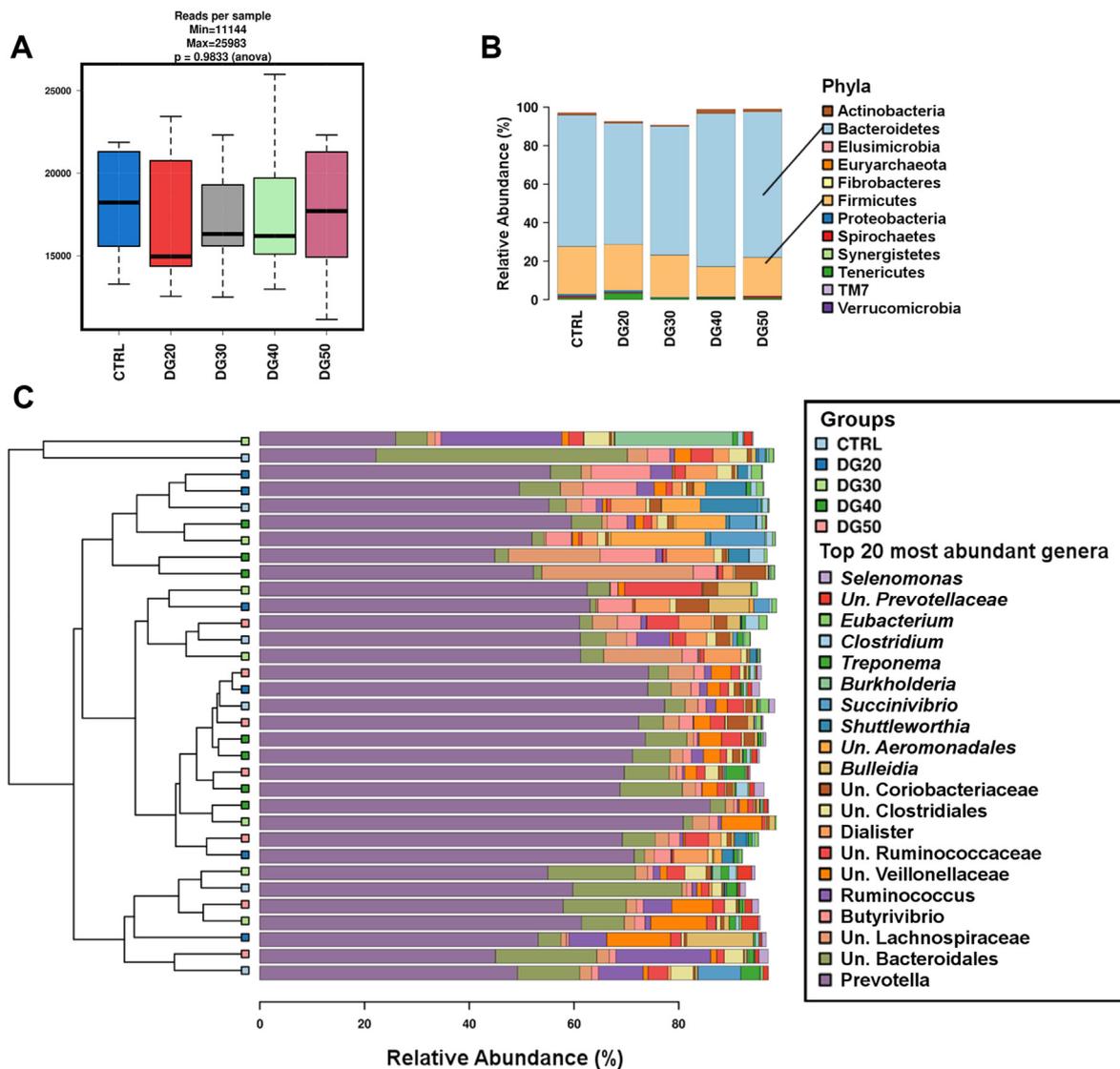


Figure 1. Sequencing quality and microbial composition of rumen fluid overview. A: Sequencing quality check showing a number of sequences per sample bar-chart; B: Distribution of bacterial phyla across the treatments; C: Clustered bar-chart of the 20 most abundant genera.

clustering, however, was not confirmed at a genus level, indicating that although total *Prevotella* genus was not different between treatments (ANOVA $P = 0.26$), DDGS influenced the change in the distribution of different *Prevotella* species.

ANOVA analysis showed no difference in any of the higher taxonomic levels, including genus. Only at an OTU level, there were significant differences ($P < 0.05$) in only 36 OTUs, 22 of those were *Prevotella*.

3.6. Microbiota correlations with DDGS concentration

We investigated the correlations of microbiota with the concentration of DDGS looking for taxa significantly induced or suppressed by DDGS. Only the phylum Verrucomicrobia and its only genus in the present dataset, unclassified RFP12, and genus *Olsenella* were correlated with DDGS concentrations by being increased by DDGS ($P < 0.05$ by both Pearson and Spearman) (Figure 3).

At an OTU level and by Pearson correlation there were 7 OTUs standing out as very strongly correlated with DDGS concentration ($P < 0.05$ and $|R| > 0.5$); 6 of those were *Prevotella* OTUs and one unknown member of Veillonellaceae family; all were also significantly correlated by Spearman and significantly differential by ANOVA ($P < 0.05$) (Figure 4). When we blasted representative sequences of these OTUs against NCBI

16S Microbial Database, we found that both OTUs positively correlated with DDGS best aligned with *Prevotella ruminicola* (91% ID), while two OTUs negatively correlated with DDGS concentration were matched to *Prevotella copri* (%ID $> 93\%$) and one to *Prevotella buccae* (92% ID) and all far from *Prevotella ruminicola* (87% ID) (Figure 4). The remaining unknown Veillonellaceae matched species of multiple genera with the same %ID and could not be associated with a single genus. The OTU matching *P. buccae* was strongly affected by any DDGS contraction (Figure 4) rather than responding to different DDGS concentrations.

3.7. Network analysis of microbiota and DDGS interactions

To expand this analysis and gain insights on how these correlations may impact on the total microbial community, we looked at networks that include not only DDGS correlations with taxa but also show correlations of microbiota with one another. Since overcrowded networks are hard to investigate, we limited this pursuit to top 20 genera and top 20 OTUs. At both genus (Figure 5) and an OTU level (Figure 6), we noticed the clear separation of two clusters comprised of mutually co-dependant correlated taxa, with both clusters strongly antagonistic (negatively correlated) with one another, and further separated by DDGS supplementation.

Table 2. The effect of feeding diets containing different levels of DDGS (% fresh basis) on feed intake (FI, kg DM), live weight gain (kg), feed conversion efficiency (FCE) and slaughter parameters of growing entire Najdi male lambs.

Dietary Ingredients	Levels of DDGS [†] (% fresh basis)					P value
	0 (Control)	20 (T1)	30 (T2)	40 (T3)	50 (T4)	
Initial body weight, kg	34.29	35.60	32.66	32.73	32.83	0.687
Final body weight, kg	53.41	54.35	50.55	49.37	49.84	0.665
Total body weight gain, kg	19.12	18.75	17.89	16.64	17.01	0.917
Experimental period, d	94	94	94	94	94	
Body weight gain, g/d	203	200	190	177	181	0.914
Total feed intake, kg DM	112	113	114	111	98	–
Daily feed intake, g/d DM	1192	1202	1213	1181	1043	–
FCE [‡] , kg gain/kg DM FI	0.17	0.17	0.16	0.15	0.17	–
Rumen pH	6.374	6.135	6.452	6.178	5.885	0.189
Slaughter parameters						
Hot carcass weight, kg	25.24	25.09	24.90	25.15	24.73	0.468
Cold carcass weight, kg	24.8	24.58	24.41	24.58	21.6	0.465
Dressing% hot	48.1	46.2	49.3	50.9	49.6	0.468
Dressing% cold	47.3	45.2	48.3	49.8	43.4	0.465
Liver wt., g	849	840	841	779	807	0.958
Compartmental stomach full, kg	5.73	4.88	4.87	4.79	6.68	0.201
Intestine full, kg	2.80	2.56	2.75	2.40	2.62	0.489
Omental fat, g	567	475	727	611	728	0.809
KKCF* wt., g	310	311	588	440	444	0.134
Tail weight, kg	2.51	2.24	2.31	2.53	2.25	0.9366

[†] DDGS = Dried Distillers Grains with Solubles.

[‡] = total live weight gain (kg)/total feed Intake (kg DM).

* KKCF = Kidney knob and channel fat.

The genus-level network (Figure 5), showing correlations of the top 20 most abundant genera with DDGS, was comprised of one cluster dominated by *Prevotella* (and including beneficial *Ruminococcus*, *Selenomonas*, *Treponema* and *Burkholderia* with a number of unknown genera) predominantly negatively correlated with the second cluster (containing *Eubacterium*, *Clostridium*, *Dialister*, *Butyrivibrio*, *Succinivibrio*, *Bulleidia* and *Shuttleworthia*), that was also suppressed by added DDGS. Despite being predominantly beneficial and common members of rumen microbiota, genera from both clusters also contain human and animal pathogenic species, and the role of DDGS promoting or suppressing some of them cannot be evaluated without testing specific pathogens in controlled experiments.

The top 20 OTUs network (Figure 6) showed DDGS promoting a cluster of correlated *Prevotella* OTUs, most highly aligned (blastn vs 16S Microbial database) with *Prevotella ruminicola* and suppressing a cluster of OTUs containing *Prevotella* species that had highest alignments with *Prevotella histocolla* and *Prevotella jejuni*. This is an interesting confirmation that the above-discussed ability of DDGS to differentially promote *Prevotella* species is notable even in the top 20 most abundant OTUs.

4. Discussion

4.1. Chemical composition of the experimental diets feed intake and performance

The higher fat content of the DDGS diets is due to the relatively high content of fat of the DDGS, which was reported to be 109 g/kg DM by Belyea et al. (2004) and 73.6 g/kg DM by Böttger and Südekum (2017).

The insignificantly lower FI with the 50% DDGS diet had no effect on body weight gain or FCE. In the contrary, Castro-Perez et al. (2014) reported a tendency toward an increase in FI ($P = 0.06$), body weight gain and hot carcass weight in lambs without effect on dressing%. Other researchers, Avila-Stagnoa et al. (2013) reported a decrease in FI with the inclusion of wheat DDGS, which was associated with a decrease in average daily gain and hot carcass weight. Van Emon et al. (2012) reported that the inclusion of DDGS in the diet of finishing lambs decreased FI but had no effect on lambs' performance, FCR or carcass characteristics. Other researchers reported a linear increase in FI as the level of DDGS in the diets of finishing lambs increased from 0 to 50% replacing barley and soybean meal (Schauer et al., 2008). The inclusion of DDGS in

Table 3. Effect of dietary inclusion of graded levels of DDGS (0, 20, 30, 40, 50% fresh weight) on blood serum concentrations of total protein, glucose, triglycerides, urea-N and albumin of the growing entire Najdi male lambs.

Serum parameters	Experimental Diets (% DDGS [†] , fresh basis)					P-value
	0 (CON)	20 (T1)	30 (T2)	40 (T3)	50 (T4)	
Total protein, g/dL	10.48 ± 1.18	11.28 ± 0.89	8.44 ± 0.83	11.21 ± 1.19	9.48 ± 0.79	0.131
Glucose, mg/dL	100.1 ± 21.3	95.2 ± 15.2	82.1 ± 11.6	94.5 ± 18.5	113.3 ± 29.0	0.055
Triglycerides, mg/dL	24.3 ± 2.5	26.4 ± 2.8	23.1 ± 4.7	22.0 ± 3.0	21.0 ± 4.0	0.528
Urea-N, mg/dL	67.5 ± 12.0	58.1 ± 4.6	52.1 ± 6.5	59.0 ± 5.6	57.9 ± 3.9	0.228
Albumin, mg/dL	3.8 ± 0.2	4.0 ± 0.3	3.9 ± 0.3	4.2 ± 0.22	4.2 ± 0.3	0.695

[†] Dried Distillers Grains with Solubles.

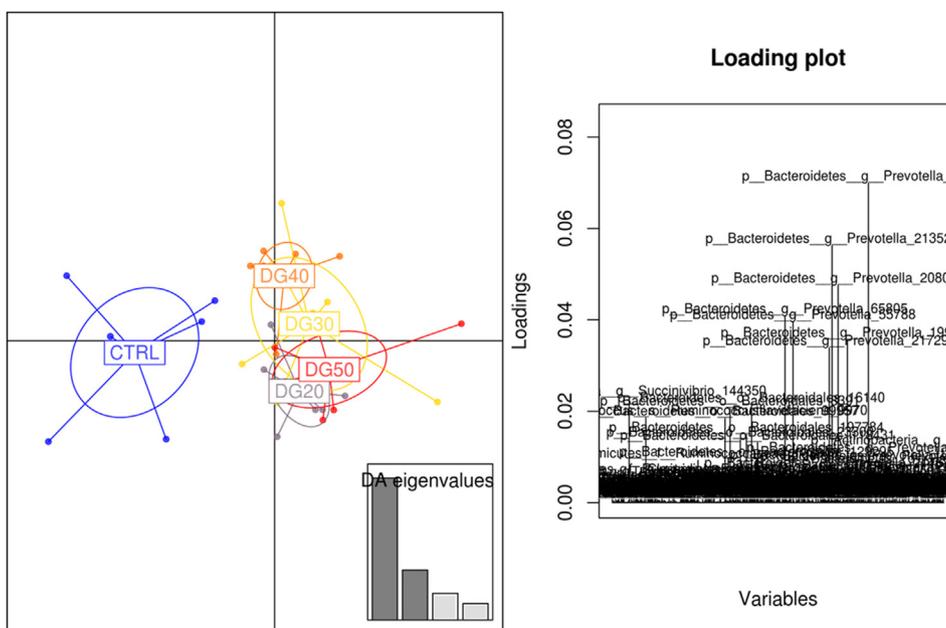


Figure 2. Discriminant Analysis of Principal Components (DAPC) plot at an OTU level.

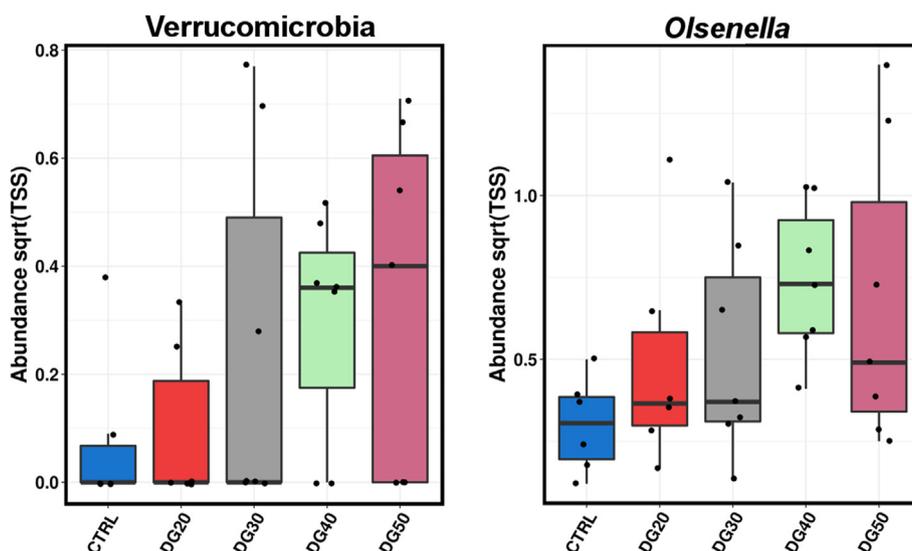


Figure 3. Correlation of taxa with the concentration of DDGS: Phylum Verrucomicrobia comprised of Unclassified RFP12 genus, and genus *Olsenella* were positively correlated with DDGS concentrations.

the finishing diets of feedlot cattle to replace grains and soybean meal is becoming increasingly popular as DDGS becoming more available and economically feasible. Gibb et al. (2008) reported that inclusion of wheat DDGS in the finishing diets of cattle at levels 20, 40, or 60% linearly increased DMI, reduced gain:feed (G:F) without affecting ADG.

Similarly, Walter et al. (2010) reported a quadratic increase in DMI with an inclusion rate of wheat DDGS but found a quadratic decrease in DMI with corn DDGS, without affecting average daily gain (ADG). Despite the volume of evidence that supports the nutritional value and safety of DDGS, it is still used with caution by animal farm managers and nutritionists. This is mainly due to the high variation in chemical composition and feeding value, particularly, ruminally undegraded feed crude protein (RUP), intestinal digestibility of RUP, and utilizable crude protein in the duodenum (Bottger and Sudekum, K.-H, 2017). Other researchers took a more cautious approach with a lower inclusion rate (5 and 10%) in the concentrate mix, partially replacing (25% and 50%) the

20% undecortecated cottonseed meal in the diet ((Omer et al., 2015) They reported an improved total body weight gain and average daily gain with moderate levels of inclusion.

McKeown et al. (2010) reported that the inclusion of DDGS improves the FCE for finishing lambs. Similarly (Gibb et al., 2008), reported improvement in FCE in cattle. Eun et al. (2009) also reported that inclusion of DDGS in the diets of growing steers at levels 10.5 (low) and 17.5 (high) improved average daily gain (ADG) and gain:feed (G:F) ratio. However, both levels of inclusions in the finishing diet of steers resulted in a similar ADG and G:F ratio (Eun et al., 2009). The positive responses of growing steers are believed to be attributed to increases in ruminal pH and aNDF digestion. However, results of the present experiment showed no differences in rumen pH. In comparison with steam-flaked corn (SFC) inclusion of corn wet distillers grains with solubles (WDGS) in the diets of yearling heifers resulted in lower gain (9%, P = 0.01), lower efficiency (7%, P = 0.01), lower hot carcass weight (3%) and 3% lower dress yield

(Depenbusch et al., 2008). One of the important chemical characteristics of distillers grains with solubles is its high-fat content, which is believed to be better than corn oil (Depenbusch et al., 2008; Eun et al., 2009; Vander Pol et al., 2009). In comparisons with 5% corn oil, wet distillers grains with solubles improved performance of yearling heifers while the 5% corn oil diet depressed G:F ratio. The finishing diet containing wet distillers grains with solubles modified rumen fermentation and resulted in higher propionate production and more unsaturated fatty acids reaching the duodenum of cattle (Vander Pol et al., 2009).

4.2. Slaughter parameters and carcass characteristics

Inclusion of DDGS in this study showed no effect on omental and KKCF fat. A similar finding was reported by Schauer et al. (2008), who reported similar performance in lambs fed the DDGS diet with no negative impact on slaughter traits. In contrast, a recent report by Curzaynz-Leyva et al. (2019) showed that lambs fed diets containing 0, 15, 30, 45% DDGS had higher hot and cold carcass weights with no adverse effect on carcass characteristics. Similarly, Buckner et al. (2007) found no differences in the slaughter parameters between steers fed diets containing 0, 10, 20, 30, 40 and 50% DDGS. While DDGS substitution did not affect dressing percentage and backfat thickness, it did linearly ($P < 0.03$) increase hot carcass weight and kidney, pelvic and heart fat (KPH), but tended to linearly decrease, as a percentage of cold carcass weight, muscle ($P = 0.08$) and insignificantly increase carcass fat (linear, $P = 0.10$) (Castro-Perez et al., 2014). Inclusion of wheat or corn DDGS in the finishing diets of feedlot steers has also shown to exhibit linear and quadratic improvement in dressing% for the wheat and corn DDGS, respectively (Walter et al., 2010). In contrast, the inclusion of DDGS in the growing and finishing diets of steers at 30% DM did not affect DMI,

ADG and G:F leading to similar final body weight and similar carcass characteristics (Leupp et al., 2009).

Szulc et al. (2010) reported a slight but not significant increase in physical fat in the carcasses of Polish Holstein bulls fattened in a two-stage feeding experiment on diets containing DDGS. For stage 1 (250–400–420 kg) the diet contained 23.5% DDGS while during the finishing stage (400–420 to 570 kg) the diet contained 11.3% DDGS. Szulc et al. (2010) performed physical dissection on the carcasses, which makes the comparison with our results difficult and indicative for potential responses only. Inclusion of DDGS in a rolled barley-based diet to a growing and finishing steers resulted in no adverse effect on performance and carcass characteristics (Eun et al., 2009). The results reported here suggest that decision for the inclusion of DDGS in the diets of growing, lactating or finishing ruminant animals will be made based on its availability and price.

4.3. DDGS induced changes in rumen microbiota structure

Multi-kingdom complex and diverse rumen microbiota, comprised of bacteria, archaea, protozoa, fungi and viral phage populations, represents the most efficient advanced natural cellulose digesting system (Weimer et al., 2010). The dominance of Bacteroidetes and Firmicutes in the rumen of Najdi lambs in this study is typical of rumen microbiota as previously described in sheep rumen by Belanche et al. (2019) and others.

Results of this study showed no change in diversity with the inclusion of graded levels of DDGS in the diets of Najdi lambs. Although it may be expected that DDGS could provide additional typical rumen desired cocktail of partially digestible structural polysaccharides, and boost the richness or influence the ratios in the bacterial community, it appears that DDGS-derived nutrients did not provide strong enough advantage to

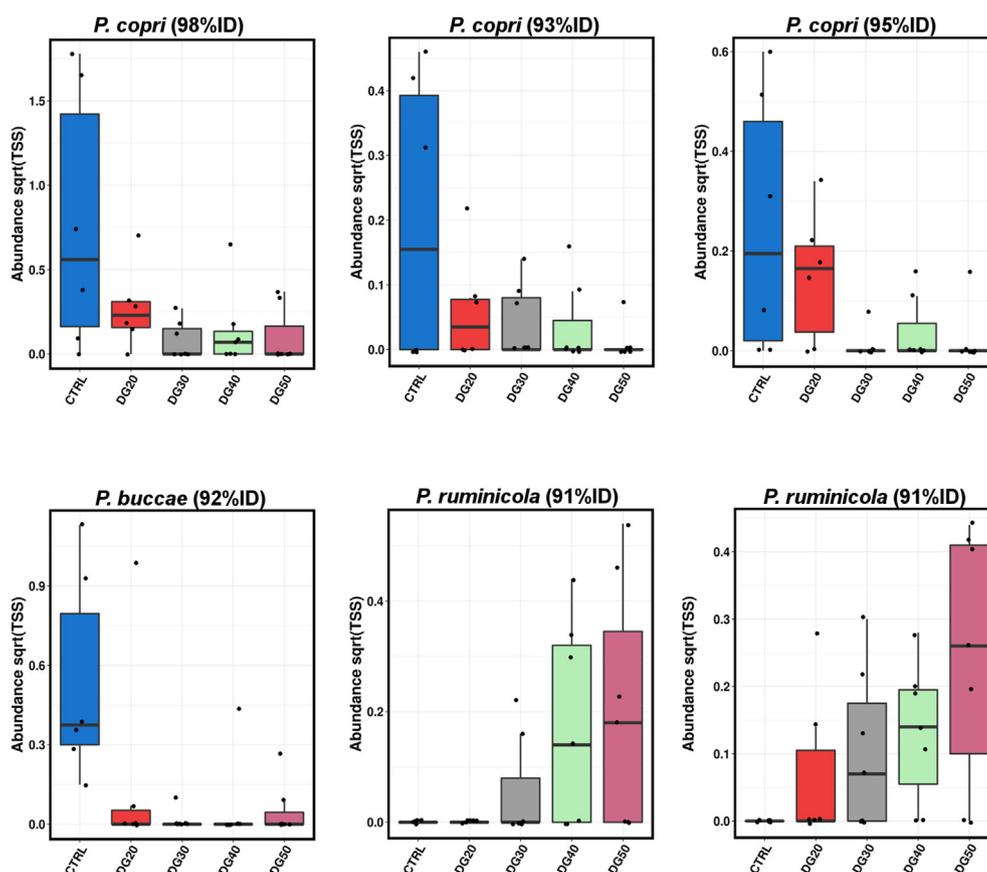


Figure 4. Correlation of taxa with the concentration of DDGS at an OTU level.

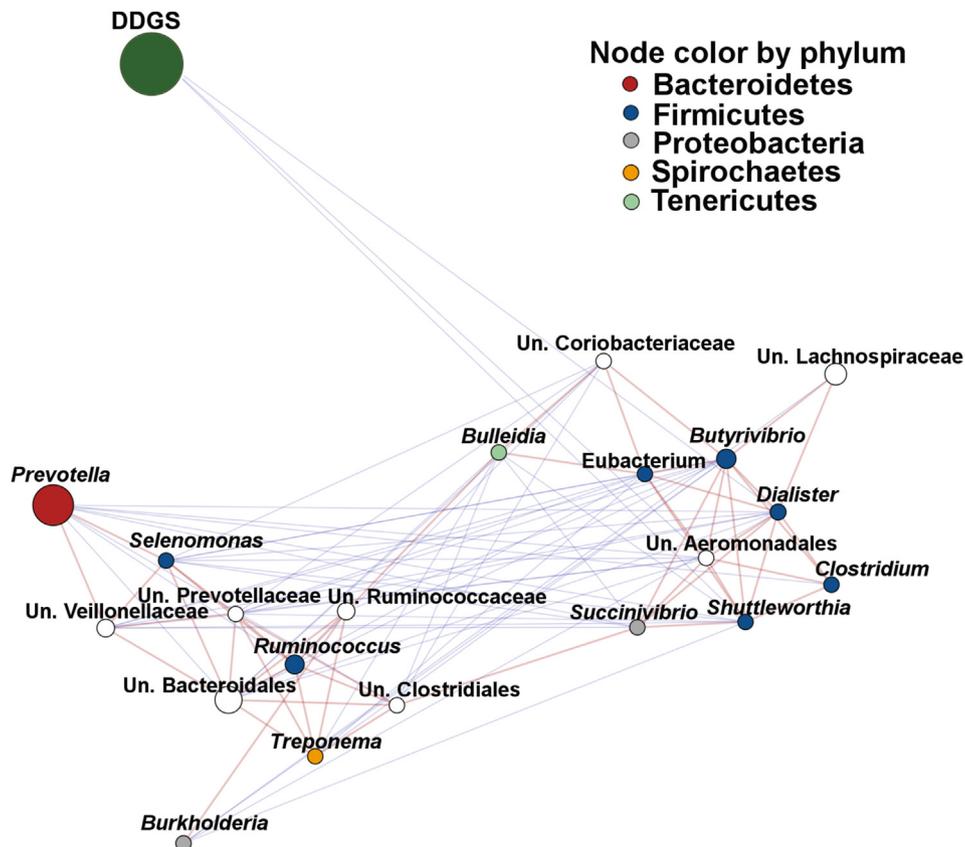


Figure 5. Genus level network presenting correlations between the top 20 most abundant genera with each other and DDGS concentrations. Red line (edge) presents a positive and blue line negative Spearman correlation. Nodes are coloured by phylum membership and sized according to relative abundance.

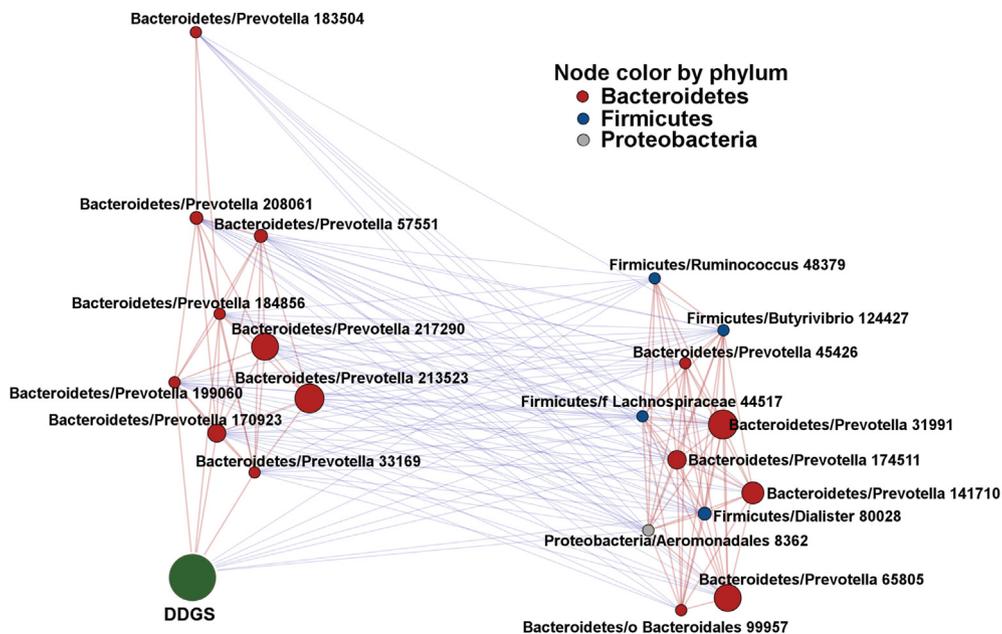


Figure 6. An OTU level network presenting correlations between the top 20 most abundant genera with each other and DDGS concentrations. Red line (edge) presents a positive and blue line negative Spearman correlation. Nodes are coloured by phylum membership and sized according to relative abundance.

boost the species that were below the detection level by sequencing method into measurable levels. On the other hand, results showed that inclusion of DDGS at the highest rate of 50% did not negatively influence the community in terms of richness or diversity.

4.4. Microbiota correlations with DDGS concentration

Similar to the impact of the dietary inclusion of DDGS on rumen microbiota structure, only one phylum, Verrucomicrobia, was affected by DDGS inclusion. Bacteria belonging to the phylum Verrucomicrobial

including members of RFP12 and *Olesenella* species are common commensal bacteria. RFP12 members are reported in horse faecal material reaching up to 38% of total sequenced reads (Steelman et al., 2012); however, they are highly unknown and unclassified in our dataset where they could be confidently assigned only to RFP12 family. *Olesenella* species are lactic acid bacteria, some species previously known as *Lactobacillus* (Dewhirst et al., 2001), identified in the healthy human mouth (Dewhirst et al., 2001), gut (Han et al., 2019), and intestinal tract of pig and sheep (Kraatz et al., 2011).

Results of this study showed that a cluster of OTUs aligned with *Prevotella* were either positively affected by the inclusion of DDGS, OTU most similar to *P. ruminicola*, or negatively affected, to *P. copri*. This indicated the possibility of two different clusters of *Prevotella* OTUs, one aligned with *P. ruminicola* and the other most similar with *P. copri*; one most likely given an advantage by DDGS by being able to utilize more DDGS supplemented complex polysaccharides. Moreover, while there are no negative reports on DDGS positively correlated *P. ruminicola*, *P. copri* is a well-known candidate for the major cause of the onset of rheumatoid arthritis (reviewed in Pianta et al., 2017).

Further analysis revealed an interesting microbial interaction between the top 20 dominant genera associated with the DDGS. Two clusters, negatively correlated with each other, one dominated by *Prevotella* while the other cluster was dominated by *Eubacterium*, *Clostridium*, *Dialister*, *Butyrivibrio*, *Succinivibrio*, *Bulleidia* and *Shuttleworthia*. Some known pathogenic bacterial species belong to some genera of the second cluster, which are impacted upon by DDGS. Further investigations are needed to shed some light onto the diet-microbes and microbes-microbes interactions that play a crucial role in determining what microbial community exist in the rumen of lambs fed DDGS containing diets.

4.5. Conclusions

Dried distillers grains with solubles (DDGS) can be included in the diets of growing and finishing lambs as a substitute for barley and soybean meal, up to 50% without adverse effect on the health and growth performance, feed efficiency, slaughter parameters, carcass traits and rumen microbiota. Inclusion of DDGS in the diets of growing and finishing ruminants will become economically feasible if it becomes more affordable compared with conventional energy and protein feed sources.

Declarations

Author contribution statement

A.M. Abudabos, M.M. Abdelrahman: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R.M. Alatiyat: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. R. Aljumaah: Performed the experiments; Contributed reagents, materials, analysis tools or data.

R. Al Jassim: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

D. Stanley: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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