ENHANCING THE UTILIZATION OF PALM LEAF HAY USING *BACILLUS SUBTILIS* AND *PHANEROCHAETE CHRYSOSPORIUM* IN THE DIET OF LAMBS UNDER DESERT CONDITIONS

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Abstract

The efficiency of the bacterium *Bacillus subtilis* and white-rot fungus *Phanerochaete chrysosporium* on growth performance, blood metabolites, carcass characteristics, meat composition and nutrient digestibility of lambs fed crushed whole palm leaf hay was investigated for 105 d. In Experiment 1 (Growth experiment), twenty-four lambs (29.5 ± 1.25 kg) were divided into four feed-based treatments: (1) concentrate and wheat straw *ad libitum* (control), (2) palm leaf hay (DPL), (3) palm leaf hay supplemented with 2 g of *B. subtilis* and *P. chrysosporium* (DPL2) or (4) palm leaf hay supplemented with 4 g of *B. subtilis* and *P. chrysosporium* (DPL4). In Experiment 2 (Digestibility experiment), twelve rams, three from each treatment, were used to study nutrient digestibility using the faecal bag technique. Results of the Experiment 1 showed that the DPL4 showed greater ($P=0.049$) live-weight gain than the control. The diets containing palm leaf hay showed lower ($P=0.001$) roughage and total feed intake and higher ($P=0.001$) feed efficiency than the control. Increased serum total protein, globulin, urea-N, aspartate aminotransferase and alanine aminotransferase (ALT) levels were observed with the DPL4 ($P<0.05$). Additionally, diets containing palm leaf hay showed higher ALT levels than the control. The DPL4 increased the hot carcass weight ($P=0.006$) and dressing percentage ($P=0.036$), and the diets supplemented with *B. subtilis* and *P. chrysosporium* decreased ($P<0.05$) the tail fat and all fat levels compared with the DPL treatment. Results of the Experiment 2 showed that nutrient digestibility was lower ($P<0.05$) with the DPL and DPL2 than with the control and DPL4. Compared with the control, nutrient digestibility of DPL4 was not affected. It is concluded that replacement of wheat straw with palm leaf hay did not affect the feed utilization or animal performance of the lambs; however, supplementation with *B. subtilis* and *P. chrysosporium* at 4 g/lamb daily enhanced the growth performance, and carcass characteristics without any adverse effects on blood metabolites.

Key words: blood metabolites, date palm leaves, digestibilities, performance, wheat straw

Semi-arid and arid regions experience low rainfall, resulting in low primary production and forage quality (Ahmed et al., 2015 a; Elghandour et al., 2016 a). Under these conditions, it seems convenient to use foliage from trees and shrubs as an adequate source of food for small ruminants (Ahmed et al., 2015 b; Salem et al., 2015). Similar to many other countries, Egypt suffers from a lack of availability of feed ingredients, especially in tropical areas under desert conditions, leading to the utilization of unconventional feeds and secondary agricultural products (Khattab et al., 2013; Kholif et al., 2014). Improving the nutritive value of fibrous feed is essential before feeding (Alserisy et al., 2015; Togtokhbayar et al., 2015; Elghandour et al., 2016 b). In Egypt, large amounts of agricultural by-products are available; however, most of these products are burned, causing environmental pollution (Khattab et al., 2011; Kholif et al., 2017 a). Date palm (*Phoenix dactilifera*) is a major crop in Egypt, with an estimated population of approximately 14 million trees producing approximately 650,000 tons of dry matter (DM) annually (Shawky and El-Sharabasy, 2015). Each date palm tree produces approximately 20 kg of leaves annually, without significant utilization. Similar to most agricultural by-products, the chemical composition, low nutritive value and low digestibility of palm leaves limits the utilization of these leaves as feed for animals (Abd El Tawab et al., 2016). Date palm leaves contain approximately 5–7% crude protein (CP) in terms of DM (Medjekal et al., 2011; Abd El Tawab et al., 2016); however, some studies (Ziaei and Hosseini, 2009; Khattab et al., 2018) have reported 10.6–16.5% CP content. Moreover, date palm fronds have high levels of cellulolytic components, ranging from 42.7 to 72.4% neutral detergent fibre (NDF) (Abd El Tawab et al., 2016; Khattab et al., 2018).

In ruminants, direct-fed microbes (DFMs) offer great potential for manipulation of ruminal fermentation and enhancement of productive performance (Elghandour et al., 2015; Sallam et al., 2020). DFMs have the ability to alter ruminal fermentation and enhance nutrient digestibility, resulting in increased daily gain or milk production and enhanced feed efficiency and animal health (Elghandour et al., 2015; Sallam et al., 2020). Reduction of harmful oxygen from the rumen and prevention of excess ruminal lactate production, as well as inhibition of ruminal pathogens, modulation of the microbial balance and release of endogenous products, are proposed modes of action of DFMs (Elghandour et al., 2015; Sallam et al., 2020). We hypothesized that DFMs affect nutrient digestion and feed utilization, resulting in enhanced animal performance. Moreover, we hypothesized that replacing wheat straw by palm leaves will not affect the palatability or nutritive value of diet. Therefore, the present study aimed to evaluate the effects of the bacterium *Bacillus subtilis* and the white-rot fungus *Phanerochaete chrysosporium*, as DFMs, in the diet of Farafra lambs fed a diet based on crushed whole palm leaf hay, replacing wheat straw. Two experiments were carried out: (1) Growth experiment to determine the effects of experimental diets on the feed intake, performance efficiency, blood metabolites and carcass characteristics of the growing Farafra lambs, and (2) Digestibility experiment to determine the effects of experimental diets on nutrient digestibility of adult Farafra rams.

Material and methods

This study was conducted at the Department of Animal Production, Faculty of Agriculture, New Valley University, New Valley, Egypt. The area has a desert climate with rare rains and high temperature. Animals were managed in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, Champaign, IL).

Preparation of the direct-fed microbial products

Ten-millilitre cultures containing 10^8 colony forming unit (CFU)/ml standard inoculums of the *P. chrysosporium* NRRL-11460 fungus and *B. subtilis* bacterium were used separately to inoculate 2000-ml Erlenmeyer flasks containing 1000 ml of the selective medium for each strain and then incubated on a rotary shaker (150 rpm). *P. chrysosporium* and *B. subtilis* were obtained from the laboratory of microbiology, Faculty of Agriculture, New Valley University (Egypt). The incubation period differed depending on the strain used to produce the broth culture media for each of the tested strains; the cell was 10^{12} CFU/ml for *B. subtilis*, while *P. chrysosporium* was calculated as 0.06 g dry weight/10 ml. An equal volume (4000 ml) from each culture was used to inoculate a fixed weight of talc powder (2000 g), each of which was separately dried at room temperature (25°C). The inoculums were added to the talc powder in intervals. The cellulase activity of *P. chrysosporium*, *B. subtilis* and the mixture was determined as described by Lone et al. (2012) to be 75.5, 81.4 and 78.0 units per gram, respectively.

Experiment 1 (Growth experiment)

Twenty-four Farafra lambs with an average body weight of 29.5 ± 1.25 kg and 5–6 months of age were randomly assigned to four experimental groups (6 lambs per treatment) and treated according to their average initial weights. The experimental period lasted 105 d, consisting of a 15-d adjustment period followed by a 90-d experimental period. The experimental design was a completely randomized design. Lambs were offered a basal diet containing a concentrate feed mixture and ground wheat straw at 60:40 (control treatment). In the other treatments, wheat straw was replaced with crushed whole palm leaf hay without supplementation (DPL treatment), supplemented with 2 g of *B. subtilis* and *P. chrysosporium* (DPL2 treatment) or supplemented with 4 g of *B. subtilis* and *P. chrysosporium* (DPL4 treatment). Diets were formulated to meet the nutrient requirements of lambs according to the NRC (2007) plus a 10% margin. Lambs were fed a fixed amount of concentrate plus *ad libitum* forage. Adjustments were made to the diets to ensure collection of orts. The chemical composition of the ingredients is shown in Table 1. The concentrate feed mixture contained 550 g of yellow corn, 225 g of wheat bran, 200 g of soybean meal, 15 g of limestone, 5 g of premix and 5 g of sodium chloride per kg DM. Ingredients were mixed and the whole concentrate was analysed. Lamb requirements were adjusted biweekly according to changes in body weight. Diets were offered to each lamb individually at 08:00 and 16:00 h in two equal portions. Daily allocations of *B. subtilis* and *P. chrysosporium* for each lamb were provided individually in 100 g of concen-

trate before morning feeding at 08:00 h to assure intake. Samples of feed ingredients were obtained daily, composited weekly, dried at 60°C in a forced-air oven for 48 h.

Table 1. Chemical composition of the concentrate mixture, wheat straw and date palm leaves

	Concentrate feed mixture*	Wheat straw	Date palm leaves
Dry matter (g/kg wet material)	930	943	944
Organic matter (g/kg DM)	948	888	924
Crude protein (g/kg DM)	151	28	42
Ether extract (g/kg DM)	33	14	23
Non-fibre carbohydrates (g/kg DM)	543	243	204
Neutral detergent fibre (g/kg DM)	273	715	731
Acid detergent fibre (g/kg DM)	142	500	524
Lignin (g/kg DM)	30	77	147
Metabolisable energy** (Kcal/kg)	2756	1081	1068

*The concentrate feed mixture contained (per kg DM): 550 g of yellow corn, 225 g of wheat bran, 200 g of soybean meal, 15 g of limestone, 5 g of premix and 5 g of sodium chloride.

**Calculated according to Ponzenga (1985).

Blood samples were collected from all the lambs from the jugular vein monthly after the morning feeding, and the last sample was obtained at the time of slaughter. Blood samples were immediately centrifuged at 3000 ×g for 15 min, and serum samples were stored at -20°C until analysis. Stored serum samples were analysed using specific kits (Stanbio Laboratory, Boerne, Texas, USA) and a T80 UV/Vis spectrometer (PG Instruments Ltd., Lutterworth, UK) following the manufacturer's instructions. Samples were analysed for total protein, albumin, glucose, urea-N, creatinine, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels. Globulin concentration was calculated by subtracting albumin levels from the corresponding total protein levels.

At the end of the experimental period, three random animals from each group were slaughtered. Fasting body weight was recorded. Immediately after slaughter, the hot carcass weight was recorded, and the weights of the head, pelt, liver, lungs, heart, spleen, kidneys, kidney fat, omental fat, testes, trachea and heart were recorded. The weight of the gut content was computed as the difference between the full and empty weights of the whole digestive tract. Measurement (slaughter weight, hot carcass, dressing percentage, right side, left side and weights of internal organs) and classification of the carcass were conducted immediately after slaughter. Carcasses were split into left and right sides using a band saw. The left and right sides were cut into four cuts, namely, right hind quarter, right fore quarter, left hind quarter and left fore quarter, which were weighed. The empty body weight was calculated as the difference between the slaughter weight and the weight of the gut content. Brisket, flank, fillet, best rib (9–11), neck, fore rib (1–6), flat rib (7–12), sirloin and round

were obtained from the left carcass side, shoulders and were weighed. Meat, fat and bone weights were separated and calculated in the best rib area.

Experiment 2 (Digestibility experiment)

Under the same conditions in lamb trial (Growth experiment), twelve adult rams weighing 50 ± 1 kg were randomly allocated into four treatment groups to determine the nutrient digestibility of the diets used in the performance experiment using the faecal bag technique. The experiment lasted 22 d, consisting of a 15-d adaptation period followed by 7 d of sample collection. Feeds and orts also were dried before grinding through a 1-mm screen for chemical analysis. Faeces were collected daily, and 10% of the faecal matter (by weight) was dried at 70°C for 24 h, combined and ground through a 1-mm mill screen for subsequent chemical analysis. Apparent digestion coefficients of the nutrients were calculated by expressing the difference between the nutrient content in both consumed feed and faeces as a percentage of intakes.

Feed, orts, and faecal samples were ground through a 1-mm screen using a Wiley mill and analysed for DM (method ID 930·15), ash (method ID 942·05), N (method ID 954·01), and ether extract (EE) (method ID 920·39), according to the official methods of the AOAC (1997). NDF levels were determined by the procedure of Van Soest et al. (1991) with sodium sulphite. Acid detergent fibre (ADF) levels (method ID 973·18) were analysed as described by the AOAC (1997). Non-fibre carbohydrate [NFC = $1000 - (\text{NDF} + \text{CP} + \text{EE} + \text{ash})$] and OM (OM = $1000 - \text{ash}$) concentrations were calculated.

Statistical analysis

Data for total gain, carcass characteristics and composition, and nutrient digestibility were analysed using the PROC GLM procedure (SAS Inst. Inc. Cary, NC, USA) for a complete randomized design with individual animal as the experimental unit. The model included the effect of treatment. Measurements (body weight changes by difference, feed intake and conversion, and blood measurements) recorded daily or weekly were analysed as a repeated measure. The model included the effect of treatment, week, and the treatment $\times$ week interaction. Two covariance structures were considered in the REPEATED statement in PROC MIXED: compound symmetry (cs) and auto-regressive (AR(1)). The error structure with the lowest Akaike information criteria that fit statistic was selected for the model. When the treatment F -test was significant at $P < 0.05$, means were compared by applying the probability of difference option of the least squares means statement. The treatment $\times$ week interaction was non-significant (i.e., $P > 0.05$) for most of the measurements; thus, only the main effects of treatments were reported.

Results

Growth performance, feed intake and feed efficiency

The initial weights of the lambs did not differ between treatments (Table 2). The daily gains did not differ between the DPL and DPL2 treatments compared to the

control treatment; however, the DPL4 lambs showed greater ($P=0.049$) daily gain than the control lambs. With no difference in daily concentrate intake, replacing wheat straw with palm leaf hay, either with or without supplementation, decreased ($P=0.001$) roughage and total feed intake. Moreover, diets containing palm leaf hay showed greater ($P=0.001$) feed efficiency than the control treatments; the order was $DPL4>DPL2>DPL$.

Table 2. Performance, feed intake and feed efficiency of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Performance (kg)						
initial weight	30.2	29.4	29.5	28.9	2.01	0.984
final weight	45.0	44.7	45.4	45.5	2.23	0.995
daily gain (g/d)	140.2 b	145.3 ab	151.8 ab	158.2 a	4.15	0.049
total gain	14.7 b	15.3 ab	15.9 ab	16.6 a	0.32	0.045
Feed intake (g/d)						
concentrate intake	1116	1112	1112	1112	13.5	0.996
roughage intake	527 a	331 b	323 b	304 b	10.6	0.001
total feed intake	1643 a	1444 b	1435 b	1417 b	20.8	0.001
Feed efficiency (g intake /g gain)	11.72 a	9.93 b	9.46 c	8.95 d	0.130	0.001

a,b,c,d – means with different letters are significant ($P<0.05$) difference. The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

Table 3. Blood metabolites of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Total proteins (mg/dl)	6.26 b	6.07 b	5.99 b	6.66 a	0.120	0.005
Albumin (mg/dl)	2.96	3.05	2.96	3.10	0.061	0.210
Globulin (mg/dl)	3.29 ab	3.01 b	3.04 b	3.62 a	0.140	0.013
Albumin/globulin ratio	0.94 ab	1.04 a	1.03 ab	0.88 b	0.044	0.040
Cholesterol (mg/dl)	68.8	67.3	71.6	65.7	2.98	0.434
Urea-N (mg/dl)	21.5 b	20.9 b	22.7 ab	24.0 a	0.63	0.031
Creatinine (mg/dl)	0.83	0.89	0.85	0.97	0.070	0.587
Alkaline phosphatase (mg/dl)	154	169	152	170	10.6	0.293
Aspartate aminotransferase (U/l)	58.7 c	60.6 bc	66.9 ab	68.9 a	2.07	0.007
Alanine aminotransferase (U/l)	11.2 b	15.6 a	16.8 a	18.6 a	1.63	0.004
Glucose (mg/dl)	60.2	59.7	58.6	60.3	1.92	0.902

a, b, c – means with different letters are significant ($P<0.05$) difference. The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

Blood metabolites

Treatment with DPL4 increased the serum total protein ($P=0.005$), globulin ($P=0.013$), urea-N ($P=0.031$), AST ($P=0.007$) and ALT ($P=0.004$) levels compared with the levels in the control treatment (Table 3). Diets containing palm leaf hay showed greater ALT levels ($P=0.004$) than the control.

Carcass characteristics and meat composition

Without any effect on other measurements, the DPL4 treatment showed the highest hot carcass weight ($P=0.006$) and dressing percentage ($P=0.036$) compared with the control treatment (Table 4). The DPL2 and DPL4 treatments showed lower tail fat ($P=0.026$) and all fat ($P=0.005$) levels than the DPL treatment.

Table 4. Carcass characteristics of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Slaughter weight (kg)	53.9	53.5	54.7	55.5	0.71	0.392
Hot carcass (kg)	23.8 c	24.5 bc	26.4 ab	27.8 a	0.96	0.006
Dressing percentage*	44.1 b	45.8 b	48.3 ab	50.2 a	0.88	0.036
Right side (kg)	11.7	12.4	13.2	13.4	0.30	0.834
Left side (kg)	12.1	12.1	13.2	14.5	0.58	0.938
Kidneys (kg)	0.12	0.12	0.14	0.10	0.020	0.521
Kidney fat (kg)	0.19	0.18	0.18	0.10	0.011	0.887
Liver (kg)	0.78	0.69	0.93	0.63	0.041	0.342
Spleen (kg)	0.15	0.13	0.16	0.12	0.010	0.554
Lungs (kg)	0.70	0.73	0.68	0.64	0.067	0.962
Heart (kg)	0.30	0.26	0.24	0.23	0.010	0.665
Testes (kg)	0.29	0.40	0.44	0.38	0.040	0.637
Head (kg)	3.91	3.14	3.63	3.37	0.162	0.749
Feet (kg)	1.19	1.04	1.11	0.99	0.074	0.180
Caul fat (kg)	0.36	0.56	0.64	0.32	0.112	0.835
Omental fat (kg)	0.29	0.37	0.36	0.27	0.024	0.728
Tail (kg)	0.27	0.23	0.22	0.18	0.034	0.613
Tail fat (kg)	2.53 ab	3.38 a	1.81 b	2.09 b	0.249	0.026
All fats (kg)	6.35 b	8.93 a	6.13 b	5.78 b	0.189	0.005

a, b, c – means with different letters are significant ($P<0.05$) difference. The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

*Dressing percentage = hot carcass (kg)/slaughter weight (kg) $\times 100$.

The characteristics of the carcass left side and its components did not differ between treatments (Table 5). Moreover, the composition (weights of 9–11 ribs, meat, fat and bone) of the best rib area of the lambs did not differ between treatments (Ta-

ble 6). Treatments did not affect the chemical composition of the *longissimus dorsi* muscle compared with the control (Table 7).

Table 5. Carcass left side and its components from lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Left side (kg)	12.1	12.1	13.2	14.5	0.58	0.938
Neck (kg)	1.38	1.48	1.42	1.51	0.373	0.741
Shoulder (kg)	2.19	2.25	2.48	2.59	0.345	0.677
Fore rib (1–6) (kg)	0.75	0.79	0.84	1.02	0.110	0.891
Flat rib (7–12) (kg)	0.77	0.80	0.79	0.89	0.200	0.931
Best rib (9–11) (kg)	0.34	0.39	0.42	0.43	0.013	0.597
Brisket (kg)	1.02	1.09	1.16	1.22	0.311	0.891
Flank (kg)	0.35	0.35	0.37	0.37	0.033	0.935
Fillet (kg)	0.14	0.14	0.12	0.12	0.010	0.951
Sirloin (kg)	1.32	1.48	1.50	1.56	0.543	0.966
Round (kg)	3.42	3.65	3.80	3.95	0.344	0.848

The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

Table 6. Components of the best ribs (9th, 10th, and 11th) of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Best rib (9–11) (kg)	0.34	0.39	0.42	0.43	0.011	0.597
Meat (kg)	0.19	0.21	0.22	0.23	0.020	0.803
Fat (kg)	0.07	0.11	0.12	0.11	0.011	0.208
Bone (kg)	0.07	0.07	0.07	0.09	0.010	0.700

The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

Table 7. Chemical composition of the *longissimus dorsi* muscle (DM basis) of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

Item	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Moisture (% DM basis)	73.4	74.1	74.2	74.0	0.46	0.763
Protein (% DM basis)	23.7	23.8	23.9	24.3	0.91	0.245
Fat (% DM basis)	18.1	18.4	16.4	16.7	0.22	0.161
Ash (% DM basis)	4.78	4.30	3.58	4.49	0.062	0.221

The P value is the observed significance level of the F-test for treatment; SEM – standard error of the mean.

Nutrient digestibility

The digestibility of OM (P=0.001), CP (P=0.003), NDF (P=0.004), NFC (P=0.045) and total digestible nutrients (P=0.002) was lower in the DPL and DPL2

treatments than in the control and DPL4 treatments (Table 8). In contrast, no difference was detected between the DPL4 and control treatments. Supplementation with *B. subtilis* and *P. chrysosporium* at 2 g daily (DPL2 treatment) did not affect nutrient digestibility compared with their supplementation at 4 g daily (DPL4 treatment) and with the DPL treatment.

Table 8. Nutrient digestibility of lambs fed date palm leaves supplemented with *Bacillus subtilis* and *Phanerochaete chrysosporium* at different levels

	Treatments				SEM	P value
	Control	DPL	DPL2	DPL4		
Organic matter (g/kg DM)	833 a	720 b	680 b	793 a	24.5	0.001
Crude protein (g/kg DM)	737 a	557 b	588 b	708 a	21.1	0.003
Neutral detergent fibre (g/kg DM)	639 a	539 b	578 b	736 a	26.4	0.004
Ether extract (g/kg DM)	639 b	649 b	653 b	756 a	33.2	0.049
Non-fibre carbohydrates (g/kg DM)	824 a	732 b	752 ab	832 a	13.2	0.045
Total digestible nutrients (g/kg DM)	747 a	662 b	684 b	781 a	13.3	0.002

a, b – means with different letters are significant ($P < 0.05$) difference. The P value is the observed significance level of the *F*-test for treatment; SEM – standard error of the mean.

Discussion

Under semi-arid and arid conditions, trees and shrubs such as date palm can be used as an adequate source of feed for goats and sheep to reduce feed cost. However, the low nutritive value of such materials determines its nutritive value. DFMs can be used to enhance the nutritive value of date palm leaves and other agriculture by-products before feeding to animals to utilize their ability to alter ruminal fermentation and enhance nutrient digestibility, resulting in improved performance (daily gain or milk production) resulting in enhanced farmers' gain and animal health.

Chemical composition

The CP content of the date leaves was approximately 42 g/kg, which is less than the range (50 to 70 g/kg DM) reported in previous studies (Genin et al., 2004; Medjekal et al., 2011). Other studies reported a higher range (106 to 165 g/kg DM) (Ziaei and Hosseini, 2009). Pascual et al. (2000) reported that date palm leaves contain low CP content (less than 17 g/kg DM) and high crude fibre content (332 g/kg DM). Moreover, date palm leaves have high levels of cellulolytic components, containing 427 to 724 g of NDF and 254 to 523 g of ADF per kg DM (Abd El Tawab et al., 2016; Khattab et al., 2018). The variation depends on the growing season, the stage of maturity, and the part of the leaf (Medjekal et al., 2011). This chemical composition indicates the low nutritive value of date palm leaves and makes it very important to enhance the nutritive value of date palm leaves before feeding to animals, via physical and biological treatments as well as feed supplementation.

Growth performance, feed intake and feed efficiency

As expected, supplementation with *B. subtilis* and *P. chrysosporium* at a high level enhanced the daily gain of lambs by approximately 12.8% compared with the control. Others (Dicks and Botes, 2010; Abd El Tawab et al., 2020; Sallam et al., 2020) have reported a positive relationship between supplementation of ruminant diets with probiotics and animal performance. Enhancement of nutrient digestibility and improvement of microbial protein synthesis result in increased amino acid supply at the post-ruminal level with supplementation and can be considered the main reasons for increasing daily gain (Retta, 2016; Sallam et al., 2020). The proposed beneficial effects of supplementation include antagonism of pathogenic organisms via antimicrobial effects, competition for adhesion sites or nutrients, stimulation of host defence mechanisms and inhibition of bacterial toxins (Elghandour et al., 2015; Retta, 2016). Additionally, increased release of different endogenous substances, including antibacterial substances, nutrients, antioxidants, growth factors and coagulating agents, enhances performance and reduces the incidence of diarrhoea by increasing the number of beneficial microorganisms in the rumen (Kochewad et al., 2009; Retta, 2016) and enhancing animal health (Bielke et al., 2003), which could also explain the enhanced daily gain of the lambs.

The inclusion of palm leaf hay in the diets decreased roughage intake by approximately 37.2 to 42.3%, which is mainly due to the palatability and physical properties of palm leaves, such as particle size and density, as well as the high levels of lignin compared with those in wheat straw (Pascual et al., 2000). The weak effects of DFMs supplementation (i.e., DPL2 and DPL4 treatments) on feed intake compared with the effects on lambs fed palm leaves without supplementation (i.e., DPL treatment) are consistent with the results of Sallam et al. (2014), who reported that DM intake was not affected when lambs were fed diets supplemented with probiotics; however, Sallam et al. (2020) observed increased feed intake when lambs were fed diets based on peanut hay supplemented with probiotics. This discrepancy may be associated with the animal models used, growth stage, environment, administration timelines, dose, feeding time, feeding duration, feeding frequency, strains, or compositions of animal diets (Whitley et al., 2009; Khan et al., 2016).

Replacement of wheat straw with palm leaf hay enhanced feed efficiency by approximately 15.3 to 23.6%, which is a result of decreased feed intake and increased daily gain for lambs fed palm leaf hay.

Blood metabolites

Blood parameter levels were within the reference ranges reported by Boyd (1984). *B. subtilis* and *P. chrysosporium* at 4 g daily increased serum total protein, globulin, and urea-N levels, indicating enhanced nutrient utilization and suggesting unaltered kidney function and minimal protein catabolism in muscles (Hosten, 1990). Our results are consistent with the results of Guo et al. (2019) who observed that inclusion of *B. subtilis* in the diets of lactating cows exposed to aflatoxin B₁ did not affect blood parameter levels.

Diets containing palm leaf hay increased the concentration of ALT by approximately 39.3 to 66.1% with no clear reason; however, the observed concentrations

were within the normal physiological range (Stanek et al., 1992). Values within normal physiological ranges are important indicators of liver activity and function (Pettersson et al., 2008).

Carcass characteristics and meat composition

There is very little published information regarding the effects of *B. subtilis* and *P. chrysosporium* supplementation on carcass characteristics. The DPL4 treatment increased the hot carcass weight and dressing percentage, which may be associated with the increased daily gain and body weight gain of lambs fed the DPL4 diet. Similar results were observed with lambs (Abdelrahman and Hunaiti, 2008) and goats (Belewu and Jimoh, 2005) fed diets supplemented with probiotics; however, other studies observed minimal effects on weights and proportions of carcass cuts in Awassi lambs or Shami goat kids (Titi et al., 2008).

The weak effects of the treatments, either wheat straw replacement or *B. subtilis* and *P. chrysosporium* supplementation, on the internal organs and wholesale cuts are similar to results reported by Soren et al. (2013).

The minimal effects of the treatments on the weights of the 9–11 ribs, meat, fat and bone of the *longissimus dorsi* muscle may be associated with the effects of the treatments on ruminal fermentation and on the relative concentrations of volatile fatty acids (Retta, 2016). Additionally, the minimal effects on the composition of the eye muscle indicate minimal effects on lipid metabolism.

Nutrient digestibility

Animals used for the nutrient digestibility trial were different from those in the lamb trial, which may have an effect on the results. Therefore, the findings of the digestibility trial can only partially help to explain what was observed in the lamb trial. Replacing wheat straw with palm leaf hay decreased nutrient digestibility even when *B. subtilis* and *P. chrysosporium* were supplemented at 2 g/lamb daily; however, supplementation at an increased level (i.e., DPL4 treatment) overcame these negative effects. The chemical composition, especially the high fibre concentration, is the main reason for the decrease in nutrient digestibility associated with a date palm leaf-containing diet without supplementation (Pascual et al., 2000; Ziaei and Hosseini, 2009; Al-Suwaieg and Al-Yousef, 2016). Additionally, the lowered CP digestibility with the DPL and DPL2 treatments may be associated with the fact that a considerable amount of CP is associated with the fibre fraction, which reduced the availability of these proteins to animals (Ziaei and Hosseini, 2009). Date palm leaves contain high concentrations of bioactive compounds, including carotenoids, isoflavones, lignans, flavonoids, tannins, and sterols, which can negatively affect nutrient digestibility (Sharifi et al., 2015).

B. subtilis and *P. chrysosporium* at high levels enhanced nutrient digestibility compared with the digestibility in lambs fed palm leaves without supplementation, revealing the importance of supplementation with these species to enhance nutrient digestibility. The modes of action and positive effects of probiotics are generally considered to involve changes in rumen fermentation rates and patterns (Retta, 2016). Probiotics have been shown to have beneficial effects on the intestinal tract

and in the rumen (Seo et al., 2010). Additionally, the supplement contains *B. subtilis*, which has a strong inhibitory effect on gastrointestinal infection by pathogens via the production of antimicrobial agents (Seo et al., 2010). However, we did not measure the growth or activity of ruminal cellulolytic microbial populations; one can speculate that probiotic supplementation increases the ruminal cellulolytic microbial populations and stabilizes the rumen pH (Ghazanfar et al., 2015), leading to improved nutrient digestion (Retta, 2016) and synthesis of microbial proteins (Uyeno et al., 2015). Saleem et al. (2017) observed that supplementation of the diet of growing lambs with probiotics improved nutrient digestibility.

The biodelignification effects of *P. chrysosporium* cause the release of lignin from cellulose and hemicellulose, and these compounds can then be utilized by microbes as energy sources for growth and enzyme production (Febrina et al., 2017). Such conditions encourage ruminal microbes to optimally digest nutrients (Kholif et al., 2017 b). Febrina et al. (2017) observed that treatment of oil palm leaves with *P. chrysosporium* enhanced nutrient digestion by goats.

Conclusions

Under arid conditions, date palm leaves can be used as forage feed for lambs, replacing conventional forages such as wheat straw. A mixture of the *B. subtilis* *P. chrysosporium*, as DFMs at 4 g daily/lamb, can enhance the growth performance and carcass characteristics of lambs fed date palm leaves without adverse effects on blood metabolites.

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