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Influence of supplementing diet with microalgae (*Schizochytrium limacinum*) on growth and metabolism in lambs during the summer

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Abstract: The current experiment was conducted to examine the impact of a supplement of microalgae (*Schizochytrium limacinum*) on the performance, rumen fermentation, and blood metabolites in lambs during summer. Forty lambs were used in a 49-day experiment. The lambs were group-fed either a basal diet (alfalfa hay and concentrate, n = 20) or the basal diet along with 5 g/day microalgae (n = 20). Feed intakes were recorded daily and body weight (BW) was measured weekly. Overall, microalgae feeding increased ($P < 0.05$) the BW and average daily gain. There were no significant differences for average feed intake or feed efficiency ($P > 0.05$). Microalgae feeding decreased rumen pH ($P < 0.05$) and tended to reduce proportion of acetate ($P < 0.1$), while it increased total rumen volatile fatty acid concentration (8.6%; $P < 0.01$) and proportions of propionate (13.9%, $P < 0.01$) and valerate ($P < 0.01$; 26.5%) compared with the control animals. Microalgae feeding increased ($P < 0.05$) blood glucose (98.47 vs. 84.97 mg/dL) and insulin (64.14 vs. 29.26 ng/mL), whereas it lowered total cholesterol concentrations in blood (62 vs. 58 mg/dL, $P < 0.1$) compared with the control animals. The results of this study indicate that microalgae supplement influences productivity and enhances dietary energy utilization in lambs during the summer.

Key words: Microalgae, blood metabolites, lamb performance, rumen fermentation, *Schizochytrium limacinum*

1. Introduction

The combination of summer temperature and humidity limit animal production, compromise the immune system, and negatively affect animal health (1). Success in overcoming the effects of heat stress is very likely related to the heat increment of feeds (2). Thus, formulations of suitable rations via adding easily digestible energy sources (reduced fiber, increased concentrates and supplemental fat), or supplementing nutraceuticals or pharmaceuticals (rumen modifiers, direct fed microbials, probiotics, antioxidants) come under the umbrella of this approach (1). However, all these methods have had little success or have shown inconsistent results (1,2).

Currently, the use of algae meal is an active area of research examining the value of these products as an important nutritional factor. The effects of these natural supplements have been documented on the immune system (3–5), gut health (6), and overall growth rate (3,7) in a number of species. Evidence also exists that certain algae preparations can increase omega-3 fatty acids in meat (8), milk (9), and eggs (10), which has implications for human health. Research also suggests that algae may positively impact metabolism and reduce lipid concentrations in animal models (10,11).

It is now possible to take advantage of algae products for the management of animal health and performance during the hot summer months. However, there is a lack of published data in the scientific literature regarding the effects of algae in stressful situations (12–16). At this point, the current research is focused on examining a microalga (*Schizochytrium limacinum*) supplement on production parameters, rumen fermentation, and bioenergetics in growing lambs during the summer. We hypothesized that the microalga (*Schizochytrium limacinum*) would affect rumen fermentation and this would improve energetic homeostasis and the growth rate of lambs during the hottest months of summer.

2. Materials and methods

2.1. Animal care and use

This experiment was conducted at a livestock experiment station. All the scientists in the experiment are licensed to perform experiments on animals and the protocol was approved by the Ethics Committee of UÜHADYEK (approval date: 23.05.2014; no: 2014-09/05).

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2.2. Animals and experimental design

Forty male Merino lambs averaging 5 months old (born in February) with body weight of 39.1 ± 1.28 kg were used in a trial conducted between 8 July and 26 August 2014, which is a specific time period when the temperature humidity index (THI) is at maximum level. Temperature and relative humidity inside the barn were measured by Data Logger (ebro, EBI 20, ebro Electronic GmbH & Co. KG, Ingolstadt, Germany) every hour on a daily basis over the duration of the experiment. The maximum, minimum, mean THI, and the THI at 1650 h were calculated by the following equation (17): {THI = (1.8 × dry bulb temperature (°C) + 32) – [(0.55 –0.0055 × relative humidity) × (1.8 × dry bulb temperature (°C) – 26.8)]}. At the beginning of the study, the lambs were randomly assigned to one of two treatments based on their body weights. Thereafter, the lambs were randomly split into 4 replicates (5/pen) for each treatment and fed diets of either control ration (n = 20) or a ration with microalgae (n = 20, *Schizochytrium limacinum*; Alltech, Inc., Nicholasville, KY, USA) for 49 days. There was a –7-day adaptation period before the experimental period. The powder form of supplement was added to the basal diet to provide product 5 g/head per day. All pens were fed with assigned ration once daily (0830) with the diet based on wheat grain and sunflower meal plus alfalfa hay. Amounts of feeds offered and refused were weighed at each pen-unit and recorded daily to calculate the feed intake. The amounts offered were adjusted according to the amounts refused; if there was <5% (on a DM basis) left for three consecutive days, the amounts were increased by 5%. However, if the amounts refused exceeded 5% for 2 days, the level of feeding was reduced by 5%. The lambs were weighed at weekly intervals throughout the trial. Average daily gain (ADG) was determined by dividing the weight gain (final live weight – initial live weight) by the number of days during each period. Skin surface temperatures were measured each day at 1650 at the left paralumbar fossa with an infrared thermography gun (Raynger MX model RayMX4PU Raytek C, Santa Cruz, CA, USA). Respiration rates were measured each day at 1650 by counting number of breaths (bpm).

Samples of the dietary rations and refusals (supplemented and unsupplemented) were collected weekly and were frozen (–20 °C) for pending analysis. Analysis was carried out according to the standard procedures of the AOAC (18). The NDF and ADF contents were determined by sequential procedures (19). Lambs had free access to water and salt licks (Na 39.3%, Cl 60.7%). The chemical composition of the ration and fatty acids profile of the diet and the supplement are given in Tables 1 and 2, respectively. Fatty acid methyl esters were analyzed using a gas chromatograph (Agilent GC system 6890N) equipped with a capillary column (Agilent, Santa Clara, CA, USA).

Rumen liquor samples were taken from animals at the beginning of the treatment period (day 0) and on the last day (day 49) of the experimental period using a stomach tube and filtered through cheesecloth. Rumen pH was measured immediately using a digital pH meter (Sartorius PB-20, Germany). Thereafter, ammonia-N was determined from 25 mL of the extract in a Kjeltech autoanalyzer without a digestion step (18). Samples were acidified with H_2SO_4 and stored at –20 °C for measuring volatile fatty acids (VFAs). The VFAs were determined in a gas chromatograph equipped with a capillary column (Stabilwax-DA; Crossbond "Carbowax"-PEG for acidic compounds, 30 m, 0.35 mm ID, 0.25 μm df, max. prog. temp. 260 °C, min. bleed at 250 °C).

Blood samples were collected from individual animals at the beginning of the treatment period (day 0) and on the last day (day 49) of the experimental period from the jugular vein into heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was harvested following centrifugation at 2200 × *g* and 4 °C for 10 min, and subsequently stored in microtubes at –20 °C until analysis. Plasma was analyzed for glutathione peroxidase (GPx, Cayman Chemicals, Cat no. 703102, USA), diamine oxidase (DAO, NeoBiolab, Cat. no. SD0039, Cambridge, USA), insulin (NeoBiolab, Cat. no. SI0011, Cambridge, USA), lipopolysaccharide binding protein (LPS-BP, Cat. no. SL0216, Cambridge, USA), free malondialdehyde (MDA, USCN, Cat. no. CEA597Ge, Houston, USA), and nonesterified fatty acids (NEFA, Eastbiopharm, Cat. no. CK-E90958, Hangzhou, China) by enzyme-linked immunosorbent assay (ELISA) (ELX808IU Ultra Microplate Reader, BIO-TEK Instruments, INC) according to the manufacturer's instructions. Biochemical parameters in plasma for glucose (Randox, Cat. no. GL 1611, United Kingdom), total protein (Randox, Cat. no. TP 4001, United Kingdom), and plasma urea nitrogen (PUN, BioAssay Systems, Cat. no. DIUR-500, CA, USA) were measured enzymatically by an automatic spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) using commercially available kits.

2.3. Statistical Analyses

Data on feed intake and feed efficiency were analyzed with pen as the experimental unit while performance traits and blood parameters were analyzed with lamb as the experimental unit. Daily measurements were condensed weekly averages for analysis. Adaptation period data were included as a covariate in the analysis. The effects of treatment on pen feed intake, skin temperatures, and heart rate were analyzed with the repeated measures with the PROC MIXED procedure of SAS 9.4 (20) with week as the repeated effect. Performance traits and blood parameters were also analyzed using the PROC MIXED procedure of SAS 9.4 (20). The results are reported as least squares

Table 1. Ingredient formulation and chemical composition of the basal diet and the microalgae.

Item	Basal diet	Microalgae
Ingredient, (%)		
Alfalfa hay	10	
Corn	15	
Wheat	47.6	
Sunflower meal, 33%	25.0	
Limestone	1.10	
Salt	1.10	
Vitamin/mineral premix†	0.15	
Chemical composition, (% of DM*)		
Dry matter	89.12	90.00
Crude protein	16.94	17.50
Organic matter (OM)	86.72	83.50
Ether extract	2.72	5.0
Crude fiber	-	7.0
ADF§	22.9	-
NDF‡	44.2	-
ME (Mcal/kg DM) ††	2.49	-

†Vitamin-mineral premix (supplied per kg): vitamin A (50,000 IU), vitamin D3 (13,300 IU), vitamin E (13,300 IU), calcium (100 g), phosphorus (67 g), sodium (20 g), magnesium (19 g), iron (3 g), copper (0.1 g), manganese (8 mg), zinc (1 g), cobalt (0.1 g), iodine (0.2 g), selenium (0.005 g)
*Dry matter; §Acid detergent fiber; ‡Neutral detergent fiber; ††Metabolizable energy estimation based on NRC (2001)

means in all cases and differences among means were declared as significant at $P < 0.05$, whereas trends were discussed at $P < 0.10$, unless stated otherwise.

3. Results

3.1. Temperature humidity index and physiological parameters

During the experimental period (49 days), mean weekly ambient temperatures in the barn ranged from 27.31 to 29.20 °C and the mean weekly THI ranged from 76.01 to 78.59 (Figure). At the time of 1651, skin temperatures ($P = 0.55$) and respiration rates ($P = 0.85$) were not affected by the supplementation (Table 3).

3.2. Growth performance

Table 3 shows the effect of microalgae supplementation on growth performance in Merino male lambs during summer. The lambs weighed 39.1 ± 1.28 kg at the start of the experiment. Initial body weights were similar for both diets ($P = 0.89$; 40.62 kg). Lambs fed microalgae had higher final body weights ($P < 0.05$; 1.64%), daily weight gain ($P < 0.01$; 29.3%), and growth rate ($P < 0.01$) than those fed the control diet. On the other hand, feed consumption ($P = 0.24$; 1256 g/day), OM ($P = 0.64$; 1231 g/day), ether extract ($P = 0.64$; 39 g/day), and CP ($P = 0.63$; 245 g/day) intakes were not influenced by the supplementation (Table 3).

3.3. Rumen fermentation parameters

Lambs fed microalgae had a higher concentration of total VFA and higher proportions of propionate (8.4%; $P < 0.01$) and valerate (26.5%; $P < 0.01$), but lower ($P < 0.01$) levels of rumen pH (Table 4). Although ammonia-N concentration; the molar proportions of butyrate, isobutyrate, and isovalerate; and the acetate:propionate ratio (A:P) did not differ ($P > 0.05$) between treatments, a tendency for decreased proportion of acetate (2.81%, $P <$

Table 2. Fatty acid composition of the diet and microalgae (*Schizochytrium limacinum*) supplement (%).

Fatty acids	Basal diet, %	Microalgae, %
Tridecanoic acid (C13:0)	0.11 ± 0.01	0.16 ± 0.01
Myristic acid (C14:0)	0.04 ± 0.01	1.55 ± 0.01
Pentadecanoic acid (C15:0)	0.04 ± 0.01	0.68 ± 0.01
Palmitic acid (C16:0)	11.72 ± 0.01	21.48 ± 0.11
Heptadecanoic acid (C17:0)	0.04 ± 0.01	0.23 ± 0.01
Stearic acid (C18:0)	1.56 ± 0.01	1.06 ± 0.01
Arachidic acid (C20:0)	0.36 ± 0.01	<0.1
Behenic acid (C22:0)	0.18 ± 0.01	<0.1
Tricosanoic acid (C23:0)	0.04 ± 0.01	<0.1
Lignoceric acid (C24:0)	0.04 ± 0.01	<0.1
SSFA	14.11	25.56
Myristoleic acid (C14:1)	0.04 ± 0.01	<0.1
Pentadecenoic acid (C15:1)	0.04 ± 0.01	<0.1
Palmitoleic acid (C16:1)	0.27 ± 0.01	0.13 ± 0.02
Heptadecenoic acid (C17:1)	0.04 ± 0.01	<0.1
Oleic acid (18:1 n9)	27.24 ± 0.12	6.61 ± 0.06
Eicosapentaenoic (C20:1 n9)	0.72 ± 0.01	3.46 ± 0.02
Erucic acid (C22:1 n9)	1.02 ± 0.01	<0.1
Nervonic acid (C24:1 n9)	0.04 ± 0.01	1.30 ± 0.01
SMUFA	29.39	11.90
Linoleic acid (C18:2 n6)	54.04 ± 0.25	8.60 ± 0.06
α-Linolenic acid (C18:3 n6)	2.30 ± 0.02	0.34 ± 0.01
Arachidonic acid (C20:4 n6)	0.04 ± 0.01	0.98 ± 0.01
Eicosapentaenoic acid (C20:5 n3)	0.04 ± 0.01	0.10 ± 0.01
Docosadienoic acid (C22:2 n6)	0.04 ± 0.01	14.0 ± 0.20
Docosahexaenoic acid (C22:6 n3)	0.05 ± 0.01	38.52 ± 0.02
SPUFA	56.50	62.54

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

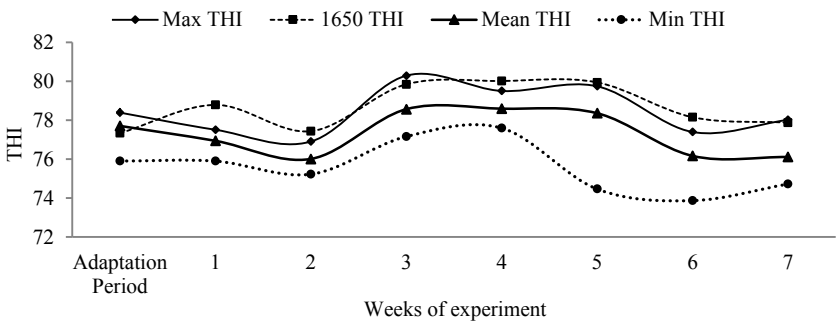

Figure. Variations in maximum, 1650, mean, and minimum temperature-humidity index (THI) over the trial period.

Table 3. Effects of dietary supplement of microalgae on performance parameters in growing male lambs.

Parameter	Control (n = 20)	Microalgae (n = 20)	SEM	P-value
Initial weight (kg)	41.55	39.69	0.32	0.89
Final weight (kg)	50.73	51.56	0.26	0.03
Total weight gain (kg)	9.18	11.87	0.94	<0.01
Daily gain (g)	187.58	242.60	12.96	<0.01
FE (g/g)	0.145	0.176	0.01	0.23
Growth rate (%/day)	0.45	0.61	0.07	<0.01
Nutrient intake (g/lamb per day)				
DM	1271.80	1240.80	17.86	0.24
OM	1240.62	1221.07	29.03	0.64
Ether extract	39.31	38.69	0.10	0.64
CP	236.3	232.5	5.53	0.63
Temperature indices				
Skin temperature (°C)	39.3	39.2	0.2	0.55
Respiratory rates (count/min)	80	76	2.0	0.85

SEM, Standard error of the mean; DIM, Dry matter intake, FE, Feed efficiency (daily gain, g/dry matter intake, g); DM, Dry matter; OM, Organic matter; CP, Crude protein

Table 4. Effects of dietary supplement of microalgae on ruminal VFA variables and ammonia-N concentration in growing male lambs.

Parameter	Control	Microalgae	SEM	P-value
pH	6.92	6.80	0.08	0.03
Total VFA, mm/L	97.70	106.14	3.08	<0.01
VFA, mol/100 mol				
Acetate (A)	60.70	58.77	1.50	0.07
Propionate (P)	19.69	22.43	0.92	<0.01
Butyrate	14.76	13.03	0.45	0.45
Isobutyrate	1.18	1.37	0.52	0.24
Valerate	2.15	2.72	0.06	<0.01
Isovalerate	1.52	1.69	0.86	0.85
A:P	3.10	2.71	0.05	0.12
Ammonium-N (mg/100 mL)	15.49	17.41	1.09	0.17

SEM, Standard error of the mean

0.10) was observed with the addition of microalgae (Table 4).

3.4. Blood metabolites

Overall, blood insulin (64.14 vs. 29.26 ± 7.50 ng/mL) and glucose (98.47 vs. 84.97 ± 1.72 mg/dL) concentrations increased ($P < 0.01$) while blood cholesterol tended to decrease (5.6%, $P < 0.10$) in lambs fed microalgae (Table 5). On the other hand, blood plasma NEFA, total protein, PUN, LPS-BP, MDA, DAO, and GPx concentrations did not differ ($P > 0.05$) between lambs supplemented with microalgae and the control group (Table 5).

4. Discussion

Seasonal high temperatures in subtropical, tropical, and arid areas are known to reduce productivity in growing animals (1,5). Therefore, the present study illustrates the impacts of heat stress in animals during hot climate conditions. The mean THI was above 76 throughout the trial, which suggests that lambs were reared in heat stress conditions (Figure) and also the respiration rate average was 78 (bpm) and skin temperature was around 39 °C (Table 3). Regardless of supplementation, this study indicates that lambs respond to ambient temperatures (21). The addition of microalgae did not affect skin temperature or respiration rate ($P > 0.05$). However, in a number of similar studies on lambs, core body temperature was decreased by supplementing different algae products during heat stress (12,15,16).

In the current study, without influencing the intake, the microalga *Schizochytrium limacinum* enhanced final mean body weights and daily weight gain by 1.64% and 29.3%, respectively (Table 3). Higher rates of weight gain in lambs (3) were observed during the summer grazing period. Yet, unaffected feed intake in cattle (22) and in lambs (23) was also observed in response to algae supplementation. The majority of the increase in production can probably be explained by the effects of microalgae on ruminal VFAs, which led to changes in ruminal fermentation (Table 4). These observations suggest that microalgae modify the ruminal microbial population (9,24). In agreement with our findings, other studies also indicate that microalgae rich in docosahexaenoic acid (DHA, 22:6n−3) affect rumen fermentation towards propionate (9,25) and isovalerate (9). The rumen pH was lower in algae-fed lambs than in control animals. This may be related to the higher concentration of ammonia and total VFA observed in this group (14). Conversely, Hopkins et al. (8), Meale et al. (23), and Clayton et al. (7) observed that the same strain of microalga (*Schizochytrium limacinum*) did not influence the growth performance or carcass traits of lambs. Treatment with the same strain of microalga in cows (26) and a brown alga (*Ascophyllum nodosum*) in steers (12) showed a reduction in feed intake under heat stress. On the other hand, Spiers et al. (12) speculated that the algae might influence metabolic heat production by decreasing intake. The reasons for the discrepancies between the present case and the others are not clear but differences in type of algae and the diet or inclusion rate are a potential explanation.

We found that the microalga *Schizochytrium limacinum* increased the concentration of glucose and reduced the concentration of cholesterol in blood (Table 5). These

Table 5. Effects of dietary supplement of microalgae on blood parameters in growing male lambs.

Parameter	Control	Microalgae	SEM[#]	P-value
Insulin (ng/mL)	29.26	64.14	7.50	<0.01
Glucose (mg/dL)	84.97	98.47	1.72	<0.01
NEFA (μmol/L)	239.86	245.89	7.31	0.68
Cholesterol (mg/dL)	61.68	58.22	0.50	0.09
Total protein (g/dL)	6.06	5.90	0.13	0.55
PUN (mg/dL)	24.23	23.56	0.84	0.58
LPS-BP (ng/mL)	260.72	261.14	1.47	0.89
DAO (ng/mL)	37.88	26.61	4.02	0.17
MDA (ng/mL)	12.56	11.97	0.43	0.49
GPx (μmol/min per mL)	81.22	88.30	4.45	0.44

SEM, Standard error of the mean; NEFA, nonesterified fatty acids; PUN, plasma urea nitrogen; LPS-BP, lipopolysaccharide binding protein; DAO, diamine oxidase; MDA, malondialdehyde; GPx, glutathione peroxidase

results indicate that *Schizochytrium* sp. may enhance energy and lipid metabolisms. PUFA sources modulate prostaglandin metabolism, lower cholesterol, and have antithrombotic and anti-inflammatory properties (27). The *Schizochytrium* sp. used in the current study was determined as an excellent PUFAs source (62.5% of total acids, Table 2). In agreement with our findings, Kannan et al. (13,14) and Karatzia et al. (28) also measured higher blood glucose levels in goats and dairy cows fed diets containing algae during stress. The hypocholesterolemic effect of microalgae also agrees with the studies in animals (10,11). In the hot summer period animals exhibit a weakened immune system and decreased antioxidant defense mechanisms (29). It has also been understood that heat stress impairs intestinal barrier integrity and causes leaky gut, which explains its negative effects on animal health and production (30). Algae have been found to protect the body from oxidative stress and to scavenge peroxides in immune cells (3,4). The MDA, GPx activity, and LPS-BP are considered useful biomarkers to assess the extent of heat stress in blood and tissue in mammals (30–32). Plasma diamine oxidase (DAO) also has been proposed as a circulating marker for monitoring the extent of intestinal barrier injury. DAO is a highly active intracellular enzyme located in the upper part of the intestinal mucosa in humans and mammals. Normally the DAO exists in very small amounts in the circulation. The DAO in the intestinal lumen is inhibited from entering the circulation by normal healthy mucosa. DAO is allowed to enter the peripheral circulation due to increased mucosal permeability when the intestinal barrier function is compromised (33). In this study, we observed an increase in plasma GPx activity and this may indicate microalgae supplementation has the potential to enhance the systemic antioxidant status of animals and helps to detoxify free radicals due to heat exposure (34). Likewise, there are other reports showing increased plasma GPx in humans (31) and heat-stressed lambs (5) with algae supplementation. Gut flora, an important index of gastrointestinal health status, was found to be improved in weaned piglets given brown algae (6). Further, omega 3 PUFAs were found to be protective in the intestinal mucosal barrier of tight junction in heat-stressed rats (35). The numerical decrease in DAO in plasma indicates that microalgae supplementation may protect the intestinal mucosa from severe injury under heat stress. Recently, DHA-enriched microalgae were found to inhibit in vitro biohydrogenation of PUFAs in the rumen (36), suggesting that higher amounts of these fatty acids can reach the small intestine. The *Schizochytrium* sp. used in the current study was shown to be a DHA (38.52% of total acids, Table 2) provider. However, it should be taken into account that the extent of PUFAs flowing to lower part of the GI tract is an important factor for marginal effects of microalgae supplement on intestinal barrier function under heat stress. Other stress markers (MDA, LPS-BP) remained unaffected because of microalgae supplementation, which suggests that the supplementation should be initiated before the start of heat stress or higher amounts of the supplement may be necessary to elicit a response in lambs.

We have concluded in this study that the addition of supplemental algae, *Schizochytrium limacinum*, to lamb diets in summer altered rumen fermentation, enhanced available energy for growth, and thus improved production. The major increase in performance is likely to be attributed to the property of microalgae as a rumen modifier, antioxidant stimulant, and health promoter. Obviously, additional studies are paramount to establish a relationship between microalgae and rumen microbial populations under heat stress conditions. Further studies are also needed to establish relationships between microalgae supplement and the gut well-being of animals under heat load.

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