BF-1605
[1-7]

RESEARCH ARTICLE | Indian Journal of Animal Research

Effect of Feeding Green Microalgae and *Bacillus subtilis* on Growth Performance, Blood Metabolites and Nutrient Digestibility of Beef Bulls in Arid Subtropics

Shaker B. AL Suwaiegh[1]

10.18805/IJAR.BF-1605

ABSTRACT

Background: Green microalgae and *Bacillus subtilis* have been known to promote productive and reproductive performances in ruminant species. The present study aimed to improve productive performance, nutrient digestibility and serum metabolites upon supplementation *Bacillus subtilis* and green microalgae under heat stress conditions.

Methods: Eighty crossbreed beef bulls were allocated into four groups of 20 bulls each. The first group served as control, the second group was supplemented with 13 g/head/day of *Bacillus subtilis*, the third group was supplemented with 30 g/head/day of *Dunaliella salina* and the fourth group was supplemented with both *B. subtilis* and *D. salina*. Daily weight gain and total weight gain were estimated. Blood samples were collected at every four weeks of experimental period and metabolic profiles were measured.

Result: Digestibility coefficients were improved in all treated groups compared to control. Body weight gains and feed efficiencies recorded higher value in *Bacillus subtilis* and microalgae group. The serum total protein were higher in green microalgae compared to other groups during weeks 8 and 12. Moreover, blood urea nitrogen was higher in all treatment groups compared to control at week 12. Microalgae and combination of both microalgae and *Bacillus subtilis* increased hemoglobin, red and white blood cells.

Key words: *Bacillus subtilis,* Bulls, Digestibility, Green microalgae, Heat stress, Metabolic Parameters.

INTRODUCTION

The implementation of feed additives and probiotics in animals' diets is increasing because it is an effective approach to improve productivity and health (Mohammed and Al-Hozab, 2019; Mohammed, 2022). *Bacillus* spp. are gram-positive spore-forming bacteria that are resistant to heat, cold, acid and digestive enzymes (Carlin, 2011). Although they cannot colonize the intestinal tracts of animals, they can have beneficial effects on productivity. It is a transitory microorganism of the digestive tract that is nonpathogenic to animals and capable of forming spores resistant to heat and cold that are very stable in the diet (Sanders *et al.,* 2003). *Bacillus subtilis* has been recognized as an effective probiotic in the livestock production. Numerous studies have been conducted to clarify the effects of *Bacillus* spp. supplements. Earlier researchers concluded that the effectiveness of *B. subtilis* in broilers as probiotics through attenuation of the pathogens with the improvement of beneficial bacteria in the gut and enhancement of average daily gain and feed efficiency (Jeong and Kim, 2014). Sun *et al.* (2011) concluded that the addition of *B. subtilis* improved calf growth and rumen development. Furthermore, in dairy cows, feeding *B. subtilis* improves milk yield (Sun *et al.,* 2013).

Although little is known about the mode of action to understand the beneficial effects, it has been suggested that *B. subtilis* enhance an anaerobic condition in the gastrointestinal tract due to rapid oxygen utilization resulting from fermentation (Hoa *et al.,* 2000). These effects of *B. subtilis* supplementation in dairy cows were examined using Holstein-Friesians characterized as high-yielding and high feed intake cows in cool temperate regions. These features lead to high heat production, accounting for 30% of the energy intake (Coppock, 1985). However, this high heat production makes the animals susceptible to heat stress.

As microalgae contributes many health effects to the humans and various researchers have exploited their antioxidant and polyphenolic contents in laboratory scale. In the present study, the effects of feeding *B. subtilis* or green microalgae or together on growth performance of beef bulls in arid subtropical regions were evaluated. We hypothesized that *B. subtilis,* green microalgae and their combination have an alleviating effect of heat stress induced by high

[1]Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, Kingdom of Saudi Arabia, P.O. Box 400 Al-Hassa, Saudi Arabia.

Corresponding Author: Shaker B. AL Suwaiegh, Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, Kingdom of Saudi Arabia, P.O. Box 400 Al-Hassa, Saudi Arabia. Email: shaker@kfu.edu.sa

How to cite this article: Suwaiegh, S.B.A. (2023). Effect of Feeding Green Microalgae and *Bacillus subtilis* on Growth Performance, Blood Metabolites and Nutrient Digestibility of Beef Bulls in Arid.Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1605.

Submitted: 04-11-2022 **Accepted:** 18-01-2023 **Online:** 07-03-2023

temperature humidity index in arid areas.

MATERIALS AND METHODS

Animal and management of experimental treatment

This experiment was carried out in Al-Ateeq farm of Saudi Arabia. Eighty crossbreed beef bull were used in this study with body weight 269.6-279.4 kg. Animals were divided randomly into four groups in equal numbers. The first group served as control group. The second group was supplemented with 13 g/head/day prepared *B. subtilis* PB6 product (6.6 × 10^9 colony forming units/g). The third group was supplemented with 30 g/head/day of *Dunaliella salina*. The fourth group was supplemented with both *B. subtilis* (13 g) and *D. salina* (30 g/head/day). The animals were fed on concentrate fed mixture (CFM), hay and wheat straw to give the requirement of dry matter (DM) and total digestible nutrients (TDN) for average body weight and daily gain of beef according to NRC (2000) requirements (Table 1). Experimental period lasted 120 days.

Climatic conditions

Ambient temperature and relative humidity were recorded during the experimental period. Air temperature and relative humidity were calculated monthly during the experimental period from June to October. Values of temperature-humidity index (THI) were calculated as

$$THI = (1.8 \times T + 32) - [(0.55-0.0055 \times RH) \times (1.8 \times T - 26)],$$

Where,

T = Temperature expressed as °C.

RH = Relative humidity (%) was based on Dikmen *et al.* (2008).

Temperature, humidity and temperature-humidity index in this region were shown in Table 2.

Blood sampling

About 10 ml of blood samples were collected from 5 bulls of each group at day 0, 4, 8 and 12 weeks of experimental period. The collected blood were used for determination of red blood cells, hemoglobin, hematocrit, white blood cells, neutrophils and lymphocytes in addition to biochemical metabolites.

Digestibility coefficient and nutritive values

Fecal sample was collected at the last week of the experimental period twice daily at 07:00 am and 02:00 pm directly from the rectum and then it was frozen until analysis. Fecal and rations samples were ground through 1 mm mill screen and mixed together then analyzed for DM, organic matter (OM), crude protein (CP), crude fiber (CF) and ether extract (EE) according (AOAC, 1995). Digestibility coefficients of DM, OM, CP, CF, EE and NFE were determined using acid insoluble ash (AIA) as internal marker according to Van Keulen and Young (1977). The nutritive values total digestible nutrients (TDN) and digestible crude protein (DCP) of the experimental rations were calculated.

Growth performance

Body weight (BW) of animals was recorded at the beginning of experimental period and monthly thereafter. Final BW, total gain and daily gain were calculated. Total feed intake was recorded during the experimental period. Values of daily average feed intake, gain and feed efficiency were calculated.

Statistical analysis

Data were analyzed using General Linear Model (GLM) procedure of SAS (SAS, 2004) according to the following model:

$$Y_{ij} = \mu + T_i + E_{ij};$$

Where,

Y_{ij} = The observation.

μ = The overall mean.

T_i = Effect of treatments.

E_{ij} = Standard error.

Duncan's multiple range tests were used to compare between means of the control and treated groups (P<0.05).

RESULTS AND DISCUSSION

Growth and Productive performance of beef bulls

The highest body weight gain was found in bulls received a mixture of *B. subtilis* and green microalgae when compared to control and *B. subtilis* groups, while there was no significant difference to green microalgae group (Table 3). Digestibility coefficient and nutritive value were improved in groups supplemented with green microalgae and a mixture of green microalgae and *B. subtilis* (Table 4).

Bacillus subtilis and/or green microalgae supplementation increased values of total digestible nutrients, starch value, digestible crude protein and digestible energy. The significant effects of *Chlorella vulgaris* algae or green microalgae on body health and productive

Table 1: Chemical composition (%) of ingredients on dry matter basis.

Items	Concentrate feed mixture	Hay	Wheat straw
Dry matter	88.76	91.18	90.35
Organic matter	93.79	88.66	89.05
Crude protein	15.76	16.54	1.79
Ether extract	2.39	2.11	1.12
Crude fiber	14.12	29.21	38.71
Non-free extract	61.52	40.80	47.43
Total ash	6.21	11.34	10.95

Table 2: Ambient temperature, relative humidity and temperature humidity index during experimental period inside the barn.

Months	Ambient , temperature	Relative humidity,	Temperature humidity index
June	36.44±0.42	23.22±1.10	80.41±0.37
July	38.72±0.42	28.56±1.10	83.02±0.37
August	37.42±0.42	30.22±1.10	83.08±0.37
September	32.11±0.42	36.00±1.10	78.11±0.37
Overall mean	36.17±0.86	28.25±1.85	81.16±0.56

performances were confirmed in several studies (Kholif *et al.,* 2020). It has been proved higher body weight gain and nutrient digestibility due to green microalgae or *B. subtilis* supplementation in several studies (Kholif *et al.,* 2020; Jia *et al*., 2022). This may be due to the increasing digestibility of nutrients, which may be attributed to the accumulation of large amounts of readily fermentable carbohydrates liberated by the action of the bacteria and probiotics in the bulls' rations (Mousa *et al.,* 2022). The improvement in most nutrient digestibility parameters could be attributed to *B. subtilis* and high protein content and β-carotene in green microalgae. This may improve the ruminal microbial activity and communities, thus increasing the gut health through rumen maturity by favoring microbial establishment, increasing the fiber digestion of feedstuff, reducing the fluid viscosity and ruminal ammonia and improving the concentration of volatile fatty acids in the rumen (Jiang *et al.,* 2020). *B. subtilis* have been used as beneficial supplements in farm animals to lower morbidity and mortality (Rai *et al.,* 2013), improve feeding performance (Jia *et al.,* 2018). Moreover, green microalgae provides organic acids and vitamins to stimulate the growth of lactic acid bacteria, which improve rumen metabolism by stabilizing the rumen pH, increasing the production of cellulolytic bacteria and improving anaerobiosis by scavenging the oxygen available in the rumen, as well as improving microbial protein synthesis and fiber digestibility (Bomba *et al.,* 2002).

Blood parameters

B. subtilis and green microalgae supplementation to bulls improved the hematological and immunological parameters (Fig 1). Other studies concluded that hematological profile showed an insignificant change between different groups (Justine and Oluwatosin, 2008; Al-Mufarji *et al*., 2022). This indicated the improved health status of the supplemented beef bulls. Such effects may be due to the improved synthesis of vitamin B_{12} by *B. subtilis* and higher content of green microalgae with vitamins B_1, B_2, B_3, B_6, B_{12}, E, K and D (Becker, 2007) and improved iron salt absorption by the small intestine, resulting in better hematopoiesis (LaFleur-

Table 3: Effect of *Bacillus subtilis* and green microalgae and their mixture supplementation on productive performance of beef bulls.

Attribute	Treatments					
	G1	G2	G3	G4	SEM	P Value
No. animals	20	20	20	20	xx	xx
Initial body weight, (kg)	275.86	272.78	274.67	275.9	2.88	-
Final body weight, (kg)	435.49[a]	470.38[b]	485.95[bc]	496.3[c]	4.88	0.03
Total gain, (kg)	159.63[a]	197.60[b]	211.28[bc]	220.4[c]	3.54	0.04
Daily gain, (kg)	1.33[a]	1.64[b]	1.76[bc]	1.84[c]	0.33	0.04
Daily feed intake, kg	8.85	9.25	9.36	10.1	0.18	0.75
Daily protein intake, kg	1.03	1.06	1.07	1.09	0.04	0.08
Feed efficiency	0.13a	0.14b	0.15[bc]	0.17[c]	0.02	0.03
Protein efficiency	1.09 a	1.24b	1.30[bc]	1.33[c]	0.05	0.06

a,b,c: Values with the different superscripts on the same row differ at P<0.05.

G1 = Control; G2 = *B. subtilis* (13 g/h/d); G3 = Green microalgae (10 g/h/d), G4 = Mixture of *B. subtilis* and green microalgae.

Table 4: Effect of *Bacillus subtilis* and green microalgae supplementation on nutrients digestibility coefficient and nutritive values of experimental rations

Item	Treatments					
	G1	G2	G3	G4	SEM	P Value
No. of animals	4	4	4	4		
Nutrients digestibility coefficient, %						
DM	69.34	71.88	72.1	72.4	2.41	0.98
OM	71.33[a]	72.54[ab]	73.14[b]	73.12[b]	1.75	0.03
CP	70.79[a]	71.98[ab]	72.62[b]	72.45[b]	1.11	0.03
CF	60.67	62.29	62.16	63.11	1.51	0.91
EE	71.10	72.81	72.61	72.37	1.55	0.92
NFE	71.38	72.81	73.10	73.10	1.54	0.92
Nutritive values, %						
TDN	63.16[a]	65.03[ab]	65.26[b]	66.24[b]	1.16	0.04
DCP	10.02[a]	10.59[ab]	10.78[b]	10.82[b]	0.50	0.03

a and b: Values with the different superscripts in the same row differ at P<0.05.

G1 = Control; G2 = *B. subtilis* supplementation (13/g/head/ day; G3 = Green microalgae supplementation (10 g/head/day, G4 = mixture of *B. subtilis* and green microalgae.

Brooks and LaFleur-Brooks, 2008). White blood cells are a major component of the body's immune system and are extremely important in defending the body against infections. The results of WBC count were consistent with those obtained by earlier reports (Milewski and Sobiech, 2009) who found that *B. subtilis* and green microalgae in bulls had a greater WBC count that participated in increasing lymphocyte percentages in the leukogram (Talha *et al.,* 2009).

Biochemical parameters

B. subtilis and green microalgae supplementation to bulls improved the serum biochemical parameters (Fig 2). The *B. subtilis* and green microalgae supplementation in our study increased blood total protein. This effect is expected, as protein digestibility was improved in the treated groups. The increased activity of hepatic function is suggested when green microalgae were fed (Tousson *et al.,* 2011), which resulted in higher concentration of total proteins as recorded in the present study. In fact, probiotics can synthesize protease enzymes and, thus, provide some specific amino acids that can boost microbial protein synthesis (Talha *et al.,* 2009). Furthermore, microalgae biomass microalgae contain 40-70% proteins (Becker, 2007) and is rich in proteins that compete favorably, in terms of quantity and quality, with conventional food proteins such as soybeans, eggs and fish, making the unicellular organisms a promising source of food protein (Batista *et al.,* 2013).

B. subtilis and microalgae supplementation improved energy status of beef bulls as indicated by glucose concentration. This enhancement can be associated with improved gluconeogenesis. Earlier studies have demonstrated that probiotic supplementation can improve gluconeogenesis by increasing propionate concentrations with a significant effect on glucose concentration in ruminants (Sayed, 2003). Furthermore, propionate is considered as the primary gluconeogenic volatile fatty acids used for glucose biosynthesis (Vanhatalo *et al.,* 2003). In addition, earlier reports found that the concentrations of total cholesterol and triglycerides were high in the green microalgae groups (Senosy *et al.,* 2017; Kholif *et al.,* 2020; Al-Mufarji *et al.,* 2022).

Blood urea nitrogen concentration can be used as an indicator of protein status as well as nitrogen utilization (Whang *et al.,* 2003; Mohammed *et al.,* 2019; 2020). The concentration of BUN increased as nitrogen intake increased up to a certain level and then reached a plateau. This can explain why increased nutrient density led to higher BUN

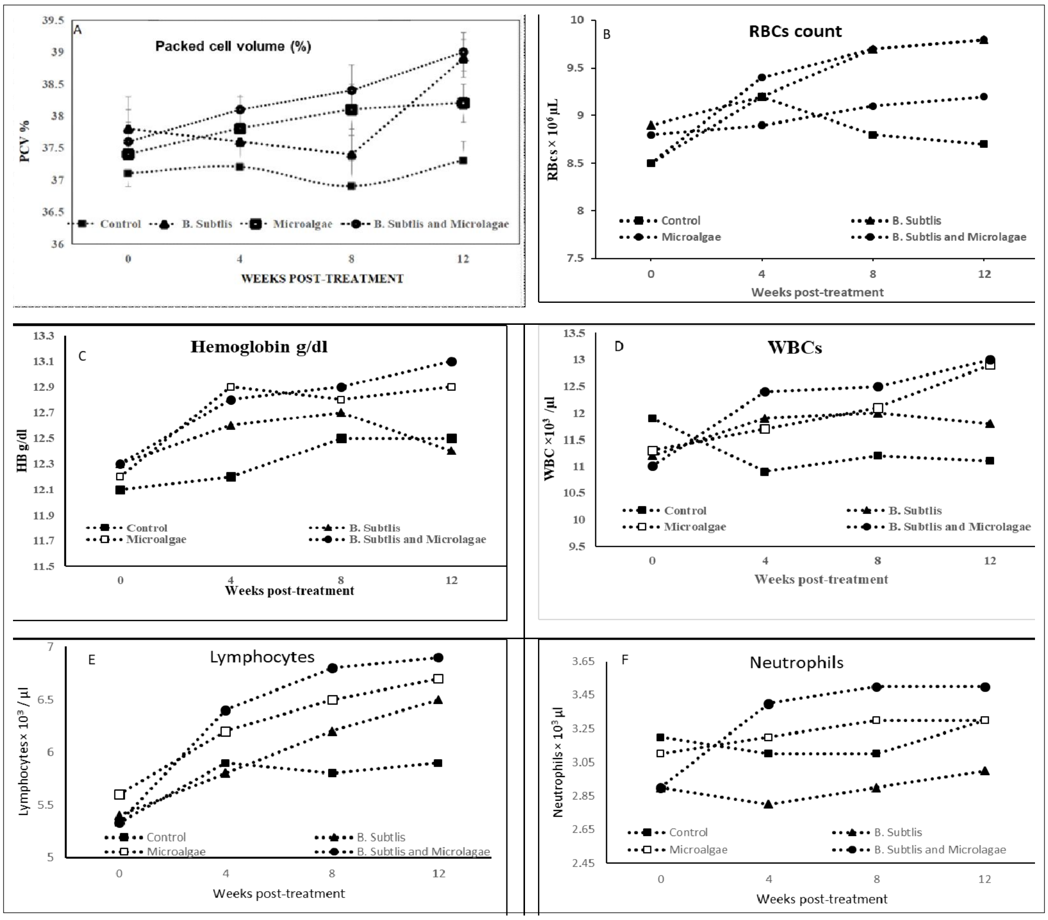

Fig 1: Effects of *Bacillus subtilis* and green microalgae supplementation on packed cell volume (A), RBCs (B) and hemoglobin (C), WBCs (D), lymphocytes (E) and neutrophils (F) concentrations.

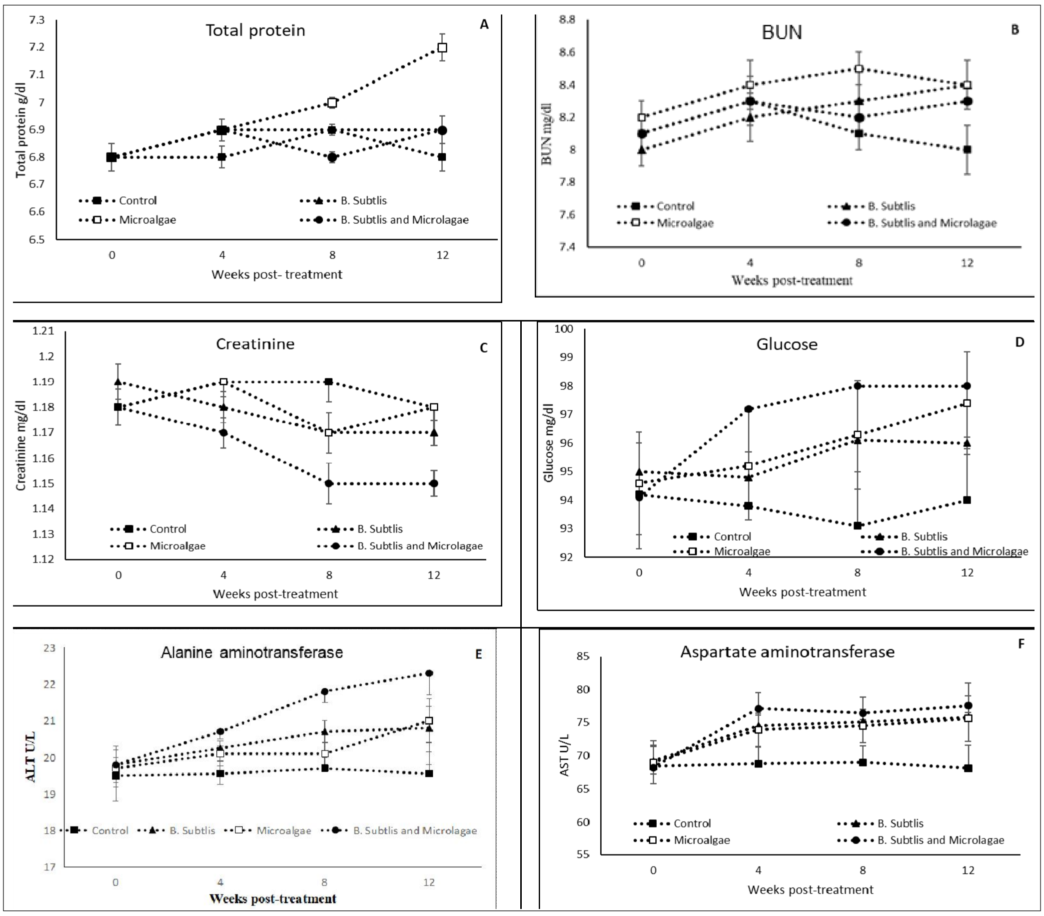

Fig 2: Effects of *B. subtilis* and green microalgae supplementation on total protein (A), blood urea nitrogen (B), creatinine (C), glucose (D), alanine aminotransferase (E) and aspartate aminotransferase (F) concentrations.

concentration in the current study. Urea nitrogen is a major waste product of protein catabolism. Our results were contrary to those of earlier researchers regarding lower blood urea nitrogen and creatinine (Senosy *et al.,* 2017; Odhaib *et al.,* 2018).

The increased AST and ALT activities may also be associated with disorders in the energy metabolism of the body as well as stress (Odhaib *et al.,* 2018). Thus, the similarity in the levels of serum AST and ALT among the treatments is an indication that the supplementation of *B. subtilis* or green microalgae had no detrimental effects on tissues and organs in beef bulls. Higher levels of AST can be a clue of liver damage, especially when paralleled with increased ALT (Pagana and Pagana, 2006). Moreover, the AST levels reported in the present study fall well within the normal bovine range (Aiello and Moses, 2019; Al-Mufarji *et al.,* 2022).

CONCLUSION

B. subtilis and green microalgae increased the nutrient digestibility, the feed intake, feed conversion, daily weight gain and total weight gain of beef bulls compared with the control. The supplementation also improved the hematological and biochemical parameters with protective effects on renal function and positive effects on energy metabolism.

ACKNOWLDEGEMENT

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (GRANT2601).

Conflict of interest: None.

REFERENCES

Aiello, S.E., Moses, M.A. (2019). Merck Veterinary Manual. Merck and Co., Inc.,White House Station, NJ.

Al-Mufarji, A., Mohammed, A.A., Al-Masruri, H., Al-Zeidi, R. (2022). Effects of dietary microalgae supplementation on mammals' production and health. Advances in Animal Veterinary Sciences. 10(8): 1718-1724.

AOAC. (1995). Official Methods of the Association Official Analytical Chemists, Washington DC.

Batista, A.P., Gouveia, L., Bandarra, N.M., Franco, J.M., Raymundo, A. (2013). Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Research. 2: 164e173.

Becker, E.W. (2007). Micro-algae as a source of protein. Biotechnology Advances. 25: 207e210.

Bomba, A., Nemcova, R., Gancarcikova, S., Herich, R., Guba, P., Mudronova, D. (2002). Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructo-oligosacharides and polyunsaturated fatty acids. British Journal of Nutrition. 88: 95-99.

Carlin, F. (2011). Origin of bacterial spores contaminating foods. Food Microbiology. 28: 177-182.

Coppock, C.E. (1985). Energy nutrition and metabolism of the lactating dairy cow. Journal Dairy Science. 68: 3403-3410.

Dikmen, S., Alava, E., Pontes, E., Fear, J.M., Dikmen, B.Y., Olson, T.A., Hansen, P.J. (2008). Differences in thermoregulatory ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. Journal Dairy Science. 91: 3395-3402

Hoa, N.T., Baccigalupi, L., Huxham, A., Smertenko, A., Van, P.H., Ammendola, S., Ricca, E., Cutting, A.S. (2000). Characterization ofBacillus species used for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders. Applied and Environmental Microbiology. 66(12): 5241-5247.

Jeong, J.S., Kim, I.H. (2014). Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gasemission and intestinal microflora in broilers. Poultry Science. 93(12): 3097-3103.

Jia, P., Cui, K., Ma, T., Wan, F., Wang, W., Yang, D., Wang, Y., Gue, B., Zhao, L., Diao, Q. (2018). Influence of dietary supplementation with *Bacillus licheniformis* and *Saccharomyces cerevisiae* as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. Scientific Reports. 8: 16712.

Jia, P., Tu, Y., Liu, Z., Li, F., Yan, T., Ma, S., Dong, L., Diao, Q. (2022). Diets supplementation with *Bacillus subtilis* and *Macleaya cordata* extract improve production performance and the metabolism of energy and nitrogen, while reduce enteric methane emissions in dairy cows. Animal Feed Science and Technology. 294: 115-481.

Jiang, B., Wang, T., Zhou, Y., Li, F. (2020). Effects of enzyme + bacteria treatment on growth performance, rumen bacterial diversity, KEGG pathways and the CAZy spectrum of Tan sheep. Bioengineered. 11: 1221-1232

Jonkers, D.M. (2016). Microbial perturbations and modulation in conditions associated with malnutrition and malabsorption. Best Practice and Research Clinical Gastroenterology. 30: 161-172.

Justine, T.E., Oluwatosin, K.Y. (2008). Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *Trypanosoma brucei* infected rats. African Journal of Biotechnology. 7: 153-157.

Kholif, A.E., Hamdon, H.E., Kassab, A.Y., Farahat, E.S.A., Azzaz, H.H., Matloup, O.H., Mohamed, A.G., Anele, U.Y. (2020). Chlorella vulgaris microalgae and/or copper supplementation enhanced feed intake, nutrient digestibility, ruminal fermentation, blood metabolites and lactational performance of Boer goat. Journal Animal Physiology and Animal Nutrition. 104(6): 1595-1605.

LaFleur-Brooks, M., LaFleur-Brooks, D. (2008). Exploring Medical Language: A Student-Directed Approach, 7th ed., Mosby Elsevier: St. Louis, MO, USA, p. 398.

Milewski, S., Sobiech, P. (2009). Effect of dietary supplementation with *Saccharomyces cerevisiae* dried yeast on milk yield, blood biochemical and hematological indices in ewes. Bulletin Veterinary Institute Pulawy. 53: 753-758.

Mohammed, A.A. (2022). Effects of copper and green algae supplementation on ovarian function, blood and metabolic profiles of Boer goats grazing copper-deficient alfalfa in arid subtropics. Fresenius. Environmental. Bulletin. 6: 6424.

Mohammed, A.A., Al-Hozab, A. (2019). +(-) catechin raises body temperature, changes blood parameters, improves oocyte quality and reproductive performance of female mice. Indian Journal Animal Research. 54: 543-548.

Mohammed, A.A., Al-Shaheen, T., Al-Suwaiegh, S. (2019). Changes of follicular fluid composition during estrous cycle: The effects on oocyte maturation and embryo development *in vitro*. Indian Journal Animal Research. 54: 797-804.

Mohammed, Al-Suwaiegh, S., A.A., Al-Shaheen, T. (2020). Effects of *myo-inositol* on physiological and reproductive traits through blood parameters, oocyte quality and embryo transfer in mice. Indian Journal Animal Research. 55: 1-6.

Mousa, G.A., Allak, M.A., Hassan, O.G.A. (2022). Influence of fibrolytic enzymes supplementation on lactation performance of Ossimi ewes. Advances in Animal Veterinary Sciences.10: 27-34.

NRC (2000). National Research Council (US). Nutrient Requirements of Beef Cattle. Seventh Revised Edition: Update 2000. The National Academy of Sciences, Washington DC.

Odhaib, K.J., Adeyemi, K.D., Ahmed, M.A., Jahromi, M.F., Jusoh, S., Samsudin, A.A., Alimon, A.R., Yaakub, H., Sazili, A.Q. (2018). Influence of *Nigella sativa* seeds, Rosmarinus officinalis leaves and their combination on growth performance, immune response and rumen metabolism in Dorper lambs. Tropical Animal Health Production. 50:1011-1023.

Pagana, K. D., Pagana, T.J. (2006). Mosby's manual of diagnostic and laboratory tests. 3d ed. St. Louis, MO: Elsevier.

Rai, V., Yadav, B., Lakhani, G. (2013). Applications of probiotic and prebiotic in animals production: A review. Environmental Ecology. 31: 873-876.

Sanders, M. E., Morelli, L., Tompkins, T.A. (2003). Spore formers as Human Probiotics: *Bacillus, Sporo lactobacillus* and *Brevi bacillus*. Comprehensive Reviews in Food Science and Food Safety. 2: 101-110.

SAS (2004). SAS user's guide: Basics. Statistical Analysis System Institute, Inc., Cary, NC, USA.

Sayed, A.S. (2003). Studies on the influence of Pronifer as a probiotic on the clinical, hematological and biochemical status of the goat's kids. Assiut Veterinary Medical Journal. 99: 131-143.

Senosy W., Kassab, A.Y., Mohammed, A.A. (2017). Effects of feeding green microalgae on ovarian activity, reproductive hormones and metabolic parameters of Boer goats in arid subtropics. Theriogenology. 96: 16-22.

Sun, P., Wang, J. Q., Deng, L. F. (2013). Effect of *Bacillus subtilis* natto on milk production, rumen fermentation and ruminal microbiome of dairy cows. Animal. 7(2): 216-222.

Sun, P., Wang, J. Q., Zhang, H. T. (2011). Effects of supplementation of *Bacillus subtilis* natto Na and N1 strains on rumen development in dairy calves. Animal Feed Science and Technology. 164: 154-160.

Talha, M.H., Moawd, R.I., Abu El-Ella, A.A., Zaza, G.H. (2009). Effect of some feed additive on rearing calves from birth till weaning:1- Productive performance and some blood parameters. Journal Animal Poultry Production. 34: 2763-2783.

Tousson, E., El-Moghazy, M., El-Atrsh, E. (2011). The possible effect of diets containing *Nigella sativa* and *Thymus vulgaris* on blood parameters and some organs structure in rabbit. Toxicology and Industrial Health. 27: 107-116.

Van keulen, J., Young, B.A. (1977) Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. Journal of Animal Science. 44(2): 282-287.

Vanhatalo, A., Varvikko, T., Huhtanen, P. (2003). Effects of various glucogenic sources on production and metabolic responses of dairy cows fed grass silage-based diets. Journal of Dairy Science. 86: 3249-3259.

Whang, K.Y., Kim, S.W., Donovan, S.M., McKeith, F.K., Easter, R.A. (2003). Effects of protein deprivation on subsequent growth performance, gain of body components and protein requirements in growing pigs. Journal of Animal Science. 81: 705-716.