Bulgarian Journal of Agricultural Science, 29 (No 6) 2023, 1137–1142

Impact of some enzymes and probiotic mixture on milk production, components and some blood traits in Awassi ewes

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Abstract

Al-Shaar, N. M., Al-Juwari, M. F., Saadi, A. M. & Almallah, O. D. (2023). Impact of some enzymes and probiotic mixture on milk production, components and some blood traits in Awassi ewes. *Bulg. J. Agric. Sci., 29*(6), 1137–1142

The study was conducted in the animals' field of the Department of Animal Production / College of Agriculture and Forestry/University of Mosul, to clarify the impact of adding a mixture of enzymes Amylase, Protease and Cellulose with the probiotic in different proportions on milk production and its components in Awassi ewes. The study was carried out by using 12 ewes by using cross over design, the ewes were divided into three groups and fed periodically on the experimental feed at three periods, and each period took up 17 days (15 days' adaptation period and two days for the collection of milk and blood samples). The ewes in all treatments were fed on standard feed formed of barley, wheat bran, soybeans and wheat straw, with a 1.5 kg/ewe per day of feed, without addition in first treatment (control), or with adding 2g from enzymes mixture and probiotic per ewe per day in the second treatment, while adding 4g of mixture enzymes and probiotic per ewe per day in third treatment. The results showed an improvement in milk production by 10% and 7%, when adding a mixture of enzymes and probiotics in the second and third treatments compared to the control, while the addition was not affected on milk components of protein, fat, lactose and solid non-fat. The results of blood measurements pointed out a significant increase ($p \leq 0.05$) in the concentration of the total protein and globulin in second treatment 7.75 and 3.51 g/dl, respectively, compared to the control treatment 6.95 and 2.7 g/100 ml, respectively, while triglycerides decreased significantly ($p \leq 0.05$) in second treatment, 38.44 mg/dl as compared to the third treatment 43.91 mg/dl. In general, the addition of the enzymes mixture and probiotic improved the production of milk and blood immune proteins in Awassi ewes.

Keywords: Probiotic; Enzymes; milk production; ewes

Introduction

The drought during the recent years resulted in lack of fodder crops available to feed the animals, this will rise the prices of feedstuffs, because of the reliance on imports to fill the deficit in the provision nutritional requirements. This situation caused a significant increase in production costs, and in order to reduce this effect, it was either use cheap feed alternatives to provide daily nutritional needs for animals, or use some food additives that can improve the efficiency of food utilization through improving the digestion, and increase milk production. This also effects the growth of lambs during the lactation period because growth absolutely depend on the amount of milk produced by their mothers and available food Hamodat (1985). The studies , which focused on improving the nutritional value of the feed and increase its digestion coefficients khjgbv and metabolism, relied on many ways, including the use of enzymes that are industrially produced and are widely in the field of nutrition, where they work alongside stomach bacteria enzymes and increase the effectiveness of decomposition in stomach(Beauchemin et al, 2003; Salem et al., 2011), in addition to Probiotics,

which consist of a mixture of bacteria and yeasts , where they provide a stable supply of lactic acid by increasing the ability of rumen bacteria to utilized and reduces their accumulation in the rumen. Yeast, also compete with the bacteria that utilized from starch and prevent the accumulation of lactic acid, thus they maintain the rumen pH, within the appropriate range for the activity of microorganism. They also exploit the oxygen in the rumen and provide a suitable environment for the work bacteria that break down fibers (Retta 2016).

Among the suggested hypotheses about the mechanism of the action of probiotics is their action as an antibiotic that helps to get rid of pathogenic bacteria in small intestine and this improves the vital system of beneficial bacteria and in turn provides greater ability for digestion and absorption Uyeno et al. (2015). All that contribute to an increase in volatile fatty acids absorbed from the rumen and an increase in microbial protein transiting into the intestine, which provides about 90% of the need for amino acids for the animal in addition to 50% of the body's need for energy Russell & Mantovani (2002), by this way, feed intake increases with better exploitation for the utilization, and improve production performance Mavrommatis et al. (2020). Some studies, pointed out that an increasing in milk production and its components could be achieved, when adding probiotics and enzymes (Al-Zubaidy, 2010; Vosooghi-Poostindoz et al., 2014), while adding enzymes and probiotics had no obvious effect in production performance in other studies (Reddish & Kung, 2007; Zilio et al., 2019). The current study was proposed to search the effect of the mixture of enzymes with the probiotics in different proportion in the diets of Awassi ewes consisting mainly of concentrated feed in milk production and its components at the beginning of milking season.

Materials and Methods

The study was conducted in a field of animals at the college of agriculture and forestry at the University of Mosul, using 12 Awassi ewes with close to birth dates. The ages of the ewe's ranges between 3–4 years and the weight range (59.97 ± 2.87). The study lasted for 51 days with crossing design experiment. The study divided into three periods, each of which lasted for 17 days, 15 days as adaptation period and the other two days for collect blood and milk samples. The ewes were divided into three groups (4 ewes of each) fed Periodically with standard diet with no additives, standard diets with an addition of 2 g of the enzymes mixture/day per ewe according the recommendations of the manufacturing company, and fed on the standard diet with the addition of 4 g of enzymes mixture/day per ewe. The enzyme mixture consists of Protease 2.750 CSU, and Amylase 5.500 SLU, Cellulase 27.5 FPUi in addition to the (*Lactobacillus acidophilus* 2.75 ×10 CFU bacteria, *Streptococcus faecium* 8.25×10 CFU bacteria, and *Bacillus subtilis* 1.1×10 CFU bacteria, which are all manufactured by (Namhyun-Dong, Gwanak-Gu, Seoul, Korea).

Ewes in each group were fed with 1.5 kg per ewe per day in two meals, first at 6 am and the second at 4 pm. The diet used consists of crushed black barley, wheat bran, straw, and some soybeans, as shown in Table 1. Milk production were recorded at the end of each period by isolating the lambs from their mothers at noon for 12 h, and then ewes milked the morning. Milk production was doubled to calculate within 24 h. Milk components were analyzed by using the milk testing device Lactosac System, Brand Name SP60 (Lohand). Blood samples were taken from the Juglar vein (10 ml) using a disposable plastic syringe for measuring (Triglyceride, urea, and blood proteins). The samples were analyzed in the laboratory by using testing kits from Biolab, a French company, and the results were read by using the scanner Biotech Engineering Management CO.LTD.UK. Data was statistically analyzed using SAS (2002), and using the cross over design on a computer.

Table 1. Components and chemical composition of basal diets

Ingredients	Percent	Chemical analysis % of dry matter	
Barley grain	50	Dry matter	91.48
Wheat bran	30	Crud protein	15.23
Soybean meal	7.25	Ether extract	2.58
Wheat straw	10	Fiber	9.59
Urea	0.75	Ash	7.78
Salt	1	Metabolizable energy, kcal/kg	2337
Limestone	1		

Chemical composition of diet was estimated according to AOAC (13), with except energy calculate from energy value of Iraqi feedstuff Al-Khawaja et al. (14)

Results and Discussion

Results in Table 2 showed that adding enzymes mixture and probiotic to the ewes' feed by 2 g per ewe daily led to insignificant improvement in milk production with a proportion, reached to 10.57%. The milk production was 878 g/day, while the improvement was 7.17%, when adding 4 g of the enzymes and probiotic mixture, milk production was 851 g/day compared to the control treatment 794 g/day. Moreover, no significant differences among the treatments in the percent of fat in milk 4.18, 3.85, and 4.22 %, protein 4.82, 4.87, and 4.81%, lactose 4.56, 4.61, and 4.55%, solid non-fat

10.38, 10.40, and 10.22% and milk energy 3577, 3623, and 3599 KJ/kg. This reflected no significant differences between the treatments in fat yield 33.29, 33.66, 36.43 g/day, protein yield 38.07, 42.59 and 40.59 g/day, lactose yield 36.05, ,40.26, and 38.50 g/day, and also in energy yield 2865, 3191, 3048 KJ/day.

Table 2. Effect of probiotic and enzyme mixture on milk yield and composition

Parameters	Control	2 g/day/ewe	4 g/day/ewe
Milk yield, g/day	794 ± 56.96	878 ± 70.09	851 ± 90.09
Milk fat, %	4.18 ± 0.19	3.85 ± 0.17	4.22 ± 0.18
Milk protein, %	4.82 ± 0.08	4.87 ± 0.09	4.81 ± 0.08
Milk lactose, %	4.56 ± 0.08	4.61 ± 0.08	4.55 ± 0.07
Milk solid non-fat, %	10.38 ± 0.14	10.40 ± 0.25	10.22 ± 0.18
Milk energy, k j/kg	3577 ± 162.48	3623 ± 111.35	3599 ± 161.83
Milk fat, g/day	33.29 ± 1.17	33.66 ± 1.41	36.43 ± 1.25
Milk protein, g/day	38.07 ± 2.99	42.59 ± 3.13	40.59 ± 2.89
Milk lactose, g/day	36.05 ± 1.48	40.26 ± 2.94	38.50 ± 1.93
Milk energy, k j/day	2865 ± 202.40	3191 ± 287.15	3048 ± 274.02
Lambs body gain, kg/lamb.	2.47 ± 0.15 b	2.57 ± 0.18 b	3.19 ± 0.21 a
Ewes body weight change, kg/ewe	-0.875	0.166	-0.791

a, b, means values within a raw with different superscripts differed ($P \leq 0.05$)

Results also showed that the total increasing of lamb's body weight significantly increased ($P \leq 0.05$) in the third treatment that fed by adding 4 g/ewe/day, it was 3.18 kg, compared to the control treatment and the treatment that fed, with the addition of 2g/ewe/day of the probiotic and mixture of enzymes, where it reached 2.47 and 2.57 kg. Ewes weight slower in the control treatment and the third treatment (-0.875 and – 0.791 kg), respectively, while it increased in the second treatment (+0.166 kg). Many studies showed that adding the probiotic to the animal's diet play a role in increasing milk production and the proportion of its components, such as protein or fat. This increase ranges between 7–25 % based on many factors, such as the probiotic type, feed type, stage production, and others Retta (2016).

Al-Zubaidy (2010) explained, that the addition of different proportions of probiotic with ratio 0.4 and 0.8% to the diets of Awassi ewes led to a significant increase in milk production with an increase in proportion of probiotic in the diet, and the reason for the improvement in milk production to the role of the probiotic in enhancing digestion, absorption and feed efficiency. Mustafa et al. (2014) pointed out that the addition of probiotic to the dairy cows' diets led to insignificant increase in milk production and the proportions of its components. Similarly, Thomas (2017) mentioned that there were no significant differences in the production of milk and its components, when feeding dairy cows with the addition of probiotic, while Satendra & Brajendu (2017) pointed out that feeding dairy cows with the addition of probiotic led to a significant increase in milk production.

Almallah (2018) mentioned that he obtained a 13% increase in the yield of Friesian cows, when bread yeast was added to the feed as a probiotic. El-Hawy et al. (2019) indicated that feeding Parki ewes on diet with the addition of probiotic led to a numerical improvement in milk production, the differences were also not significant in milk components. Mavrommatis (2020) showed that the addition of yeast as a probiotic to the diets of Awassi ewes led to an increase 7.6% in milk production. Almallah et al. (2021) did not find a significant differences in milk production and the percentage of components, when yeast was added as a probiotic to the Awassi ewes' diets. Similarly, Boushehri et al. (2021) mentioned that feeding dairy cows with the addition of antioxidants with probiotic in an amount 10 and 20 g/cow did not lead to significant differences in milk production and its components except the fat, which was significantly increased compared to the control treatment. Previous studies had agreed that the improvement of milk production with the addition of the different kinds of probiotics was due to the direct effect of probiotics in digestion and metabolism and increased availability of nutritional compounds for absorption in the intestine or indirect effect on ruminal monocarboxylate transport, which will operate with the transport system of the epithelial cells wall of the rumen and thus the absorption of the resources of milk components from volatile fatty acids Nalla et al. (2022).

On the other hand, regarding the effect of adding enzymes to ruminant diets, Yang et al. (2000) pointed out that feeding dairy cows with the addition of fibro-lytic enzymes led to improve the digestion significantly, but that did not significantly reflect in improving milk production or the proportions of its components or the components yield. This was also reached by Flores et al. (2008), when adding a mixture of enzymes cellulose and xylanase to the ewes' diet did not lead to a significant difference in milk production and its components, while Rojo et al. (2015) mentioned that adding cellulose enzymes to the diet of goats had a significant ef-

fect in increasing milk production and energy in milk, and yield of milk components. Silva et al. (2016) pointed out that there were insignificant differences in milk production and its components in the mid stage of milk production.

Reddish & Kung (2007) observed that there were no significant differences in milk production and the proportions of its components, when 10 g of a mixture of fibers enzymes were used in dairy cows' diets, the authors stated that reason for the lack of response was not clear, but it might be related to the fact that enzymes differ in their ability to analyze special types of compounds and might be had a stability degree of the rumen. Zilio (2019) use mixture of enzymes in dairy cows feeding as additive, they indicated that the reason for the lack of improvement in milk production with the addition of enzyme might be significantly affected by the stage of lactation and the energy state of the body.

The results of blood measurements in Table 3 showed that feeding the mixture of enzymes with probiotic led to increase of the total protein in the blood so for the two addition treatments 7.75 and 7.23 g/dl and that increase was significant ($P \leq 0.05$) in the treatment that fed with the addition of 2 g/ewe per day compared to the control treatment 6.95 g/dl, and that increase in total protein occurred, because of the increasing of globulin protein in those treatments, they were 3.51 and 3.05 g/dl, compared to the control treatment 2.71 g/dl with a significant increasing ($P \leq 0.05$) in treatment that fed with the addition 2 g of a mixture of enzymes and probiotic, compared to the control treatment, while the differences were not in the level of significance in the concentration of the albumin protein in the blood, 4.06, 4.23 and 4.18 g/dl, respectively. The concentration of triglycerides significantly decreased ($P \leq 0.05$) in the second treatment, when the mixture of enzymes and probiotic were added with amount 2 g/ewe 38.44 mg/dl compared to the third treatment that fed with the addition 4 g/ewe of a mixture of enzyme and probiotic 43.91 mg/dl, while the differences were not in the level of significance with the control treatment 41.11 mg/dl, results also showed that there were no significant differences between the treatments in the concentration of the cholesterol 68.00, 70.65 and 70.70 mg/dl or urea blood 30.15, 27.45 and 29.87 mg/dl, respectively.

Table 3. Effect of probiotic and enzyme mixture on some blood parameters

Blood parameters	Control	2 g/day/ewe	4 g/day/ewe
Total protein, g/dl	6.95 ± 0.25 b	7.75 ± 0.21 a	7.23 ± 0.26 ab
Albumin, g/dl	4.06 ± 0.18	4.23 ± 0.19	4.18 ± 0.11
Globulin, g/dl	2.71 ± 0.13 b	3.51 ± 0.10 a	3.05 ± 0.24 ab
Triglycerides, mg/dl	41.11 ± 1.02 ab	38.44 ± 2.03 b	43.91 ± 1.04 a
Cholesterol, mg/dl	68.00 ± 1.35	70.65 ± 1.47	70.70 ± 1.44
Urea, mg/dl	30.15 ± 0.92	27.45 ± 0.83	29.87 ± 1.09

a, b, means values within a raw with different superscripts differed ($P \leq 0.05$)

Mustafa et al. (2014) explained that feeding the probiotic with the diet did not lead to a significant difference on the blood parameters urea, total protein and globulin, but albumin increased significantly compared to the control treatment. But Dabiri et al. (2016) pointed out that protein was not affected, when adding a low, or high level of the probiotic to the diets of ewes at weaning, but the urea decreased significantly with the high level. The authors stated that the reason for that was due to the improvement in the efficiency of nitrogen utilization through activating rumen bacteria. Almallah (2018) pointed out that there was no effect in cows when adding the yeast as a probiotic in the blood traits.

The studies that have been conducted on the effect of feeding enzymes. It was indicated by Gado et al. (2014) that adding fibrolytic enzymes with vitamins to ewes' diets led to a significant increase in the concentration of the total protein. On the contrary, Peter et al. (2015) explained that there was no significant effect in the concentration of the total protein in dairy cows, when adding with regard to the studies that have been conducted on the effect of feeding enzymes, it was indicated enzymes to the feed consisting of 60% roughage and 40% concentrate feed. Al-Rubaie et al. (2018) also stated that adding fibro-lytic enzymes to the diets of Awassi ewes with proportions 0, 1, 3, 5 kg/ton from the diet led to increase in urea and cholesterol in blood, without affecting the proteins, they explained that the reason of increasing the urea in blood is the imbalance in the exploitation of rumen ammonia by rumen bacteria.

Beigh et al. (2018) stated that the fibro-lytic enzymes did not lead to a significant difference in the blood measurements compared to the control treatment, they attributed the reason for the lack of change in blood characteristics to the fact that the amount of consumed energy was sufficient to meet the needs of the animals. While Shaaban et al. (2021) mentioned that adding enzymes to the diets consisting of an olive pulp, or olive silage led to increase of globulin in blood significantly accompanied by lower cholesterol, without affecting the urea and triglycerides.

Conclusion

It is clear from the results of the study that adding a two level of probiotic to the diet of Awassi ewes in the early stage of lactation, led to improve in milk production with the percent ranged from 7–10%, while the components of milk did

not affect, also we found an increase in blood total protein due to an increase in immune proteins (globulin) in blood, and this may be correlated to the probiotic to the diets of Awassi ewes.

Acknowledgments

The authors will thank University of Mosul for the provided the requirements of work completion.

References

Al-Khawaja, **A. K., Al-Bayati, E. A. & Matti, S. A.** (1978). Chemical composition and nutritional value of Iraqi feed materials. *Ministry of Agriculture and Agrarian Reform, General Directorate of Animal Resources*.

Almallah, **O. D.,** (2018). Effect of Yeast Supplement to The Rations Differed in Degradable Protein in Milk Production and Components in Cow. *Mesopotamia Journal of Agriculture*, *46*(2), 158-165.

Almallah, **O. D., Abdullah, M. N., Abbo, N. Y. & Hussien, N. G.** (2021). Impact of yeast supplement to the rations contained different levels of barley grain in milk production and components of awassi ewes. *Asian Journal of Dairy and Food Research*, *40*(3), 295-298.

Al-Rubaie, **G. M. N., Ajeel, H. M. & Khalaf, S. S.** (2018). Effect of add exogenous fibrolytic enzymes to the ration in some characteristics of blood biochemicals of Awassi lactating ewes. *Al-Anbar Journal of Veterinary Sciences*, *11*(1).

Al-Zubaidy, A. K. (2010). Effect of different levels of Iraqi probiotic in Awassi ewes rations on milk production and lamb's growth until weaning. *Journal of Karbala University, 8*(3), 272-282.

AOAC (2000). Official Methods of Analysis. 15th end. Association of Oficial Analytical Chemists, Arlington, Virginia, 69-88.

Beauchemin, **K. A., Colombatto, D., Morgavi, D. P. & Yang, W. Z.** (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science, 81(*14_suppl_2*),* 37-47.

Beigh, **Y. A., Ganai, A. M., Ahmad, H. A., Mir, D. M., Bhat, M. A. & Muzamil, S.** (2018). Blood metabolic profile of lambs fed complete diet supplemented with exogenous fibrolytic enzymes cocktail. *J. Anim. Heal. Prod.*, *6*, 96-102.

Boushehri, M., Sadeghi, A. A., Chamani, M. & Aminafshar, M. (2021). Effects of antioxidants and prebiotics as vegetable pellet feed on productionperformance, hematological parameters and colostrum immunoglobulin content in transition dairy cows. *Italian Journal of Animal Science*, *20*(1), 1863-1869.

Dabiri, **N., Yazdi, A. B., Hemati, B., Bahrani, M., Mahdavi, A., Raghebian, M. & Hajimohammadi, A.** (2016). Effect of different levels of Biosaf probiotic in diet of late pregnant and lactating Iranian Zandi Ewes, on growth performance and immune system of their lambs. *J. Fisheries Livest. Prod.*, *4*(207), 2.

El-Hawy, **A. S., El-Bassiony, M. F., Bakr, S. A., Gawish, H. A. A., Badawy, M. T. & Gado, H. M.** (2019). Productive and reproductive performance and metabolic profile of barki ewes supplemented with two forms of probiotics as feed additives. *World*, *9*(2), 135-145.

Flores, **C., Caja, G., Casals, R., Albanell, E. & Such, X.** (2008). Performance of dairy ewes fed diets with a fibrolytic enzyme product included in the concentrate during the suckling period. *Animal, 2*(6), 962-968.

Gado, **H. M., Almustafa, S. S., Salem, A. Z., Khalil, F. A. & Abdalla, E. B.** (2014). Influence of vitamins and exogenous enzymes combination on alleviating heat stress in lactating ewes under Egyptian summer conditions. *Animal Nutrition and Feed Technology*, *14*(1), 195-203.

Hamodat, **S. G.** (1985). Effect of total milk production on the growth of suckling lambs in Hamdani sheep. Msc. Thesis, college of Agrculture, Sallaheldin University.

Mavrommatis, **A., Mitsiopoulou, C., Christodoulou, C., Karabinas, D., Nenov, V., Zervas, G. & Tsiplakou, E.** (2020). Dietary supplementation of a live yeast product on dairy sheep milk performance, oxidative and immune status in peripartum period. *Journal of Fungi*, *6*(4), 334.

Mustafa, **M. I., Mehmood, M. M., Lateef, M., Bashir, M. K. & Khalid, A. R.** (2014). Factors influencing lamb mortality from birth to weaning in Pakistan. *Pakistan Journal of Life & Social Sciences*, *12*(3).

Nalla, **K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M. & Puniya, A. K.** (2022). Impact of Probiotics on Dairy Production Efficiency. *Frontiers in Microbiology*, 1519.

Petter, A., Meyer, U. & Dänicke, S. (2015). Effect of exogenous fibrolytic enzymes on performance and blood profile in early and mid-lactation Holstein cows. *Animal Nutrition*, *1*(3), 229-238.

Reddish, **M. A. & Kung, Jr. L.** (2007). The effect of feeding a dry enzyme mixture with fibrolytic activity on the performance of lactating cows and digestibility of a diet for sheep. *Journal of Dairy Science*, *90*(10), 4724-4729.

Retta, K. S. (2016). Role of probiotics in rumen fermentation and animal performance: Review. *International Journal of Livestock Production*, *7*(5), 25-32.

Rojo, **R., Kholif, A. E., Salem, A. Z. M., Elghandour, M. M. Y., Odongo, N. E., De Oca, R. M. & Alonso, M. U.** (2015). Influence of cellulase addition to dairy goat diets on digestion and fermentation, milk production and fatty acid content. *The Journal of Agricultural Science*, *153*(8), 1514-1523.

Russell, **J. B. & Mantovani, H. C.** (2002). The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *Journal of Molecular Microbiology and Biotechnology*, *4*(4), 347-355.

Salem, **A. Z. M., El-Adawy, M., Gado, H., Camacho, L. M., Gonz**ález-**Ronquillo, M., Alsersy, H. & Borhami, B.** (2011). Effects of exogenous enzymes on nutrients digestibility and growth performance in sheep and goats. *Tropical and Subtropical Agroecosystems*, *14*(3), 867-874.

SAS (2002). SAS/STAT Software*, Version 9.2.* SAS Institute Inc., Cary, NC, USA.

Satendra, **K. & Brajendu, K.** (2017). Effect of supplementation of probiotic on the performance of milk yield and economics of dairy cows. *Journal of Animal Feed Science and Technology*, *5*, 55-60.

Shaaban, **M. M., Abd El Tawab, A. M., Hadhuoud, F. I., Eid, S. Y., Mostafa, M. M. M., El-Zaher, H. M. & El-Ganainy, S. M. M.** (2021). Olive cake and fibrolytic enzymes inclusion in ewes' diets and its effect on blood values and reproductive performance. *Adv. Anim. Vet. Sci.*, *9*(6), 879-886.

Silva, **T. D., Takiya, C. S., Vendramini, T. H. A., de Jesus, E. F., Zanferari, F. & Rennó, F. P.** (2016). Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows. *Animal Feed Science and Technology*, *221*, 35-43.

Thomas, **A. D.** (2017). Supplementation of two novel probiotics in the diet of lactating dairy cows (Doctoral dissertation, Iowa State University), 306-318.

Uyeno, **Y., Shigemori, S. & Shimosato, T.** (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, *30*(2), 126-132.

Vosooghi-Poostindoz, **V., Foroughi, A. R., Delkhoroshan, A., Ghaffari, M. H., Vakili, R. & Soleimani, A. K.** (2014). Effects of different levels of protein with or without probiotics on growth performance and blood metabolite responses during pre-and post-weaning phases in male Kurdi lambs. *Small Ruminant Research*, *117*(1), 1-9.

Yang, **W. Z., Beauchemin, K. A. & Rode, L. M.** (2000). A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *Journal of Dairy Science*, *83*(11), 2512-2520.

Zilio, E. M., Del Valle, T. A., Ghizzi, L. G., Takiya, C. S., Dias, M. S., Nunes, A. T. & Rennó, F. P. (2019). Effects of exogenous fibrolytic and amylolytic enzymes on ruminal fermentation and performance of mid-lactation dairy cows. *Journal of Dairy Science*, *102*(5), 4179-4189.

Received: **September, 28, 2022**; *Approved*: **October, 31, 2022;** *Published:* **December, 2023**