Evaluation of different portions of the ImmuPlus® polyherbal additive fed to fattening lambs

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ABSTRACT

Objective: To verify if the ImmuPlus® polyherbal feed additive (*Tinospora cordifolia*, *Ocimum sanctum*, *Whitania somnifera*, *Andrographis paniculate*, and *Azadirachta indica*) improves the productive performance of finishing lambs.

Design/Methodology/Approach: Twenty Hampshire × Suffolk male lambs (initial live weight 33.6±1.9 kg) were distributed according to a completely randomized design. Treatments consisted of the inclusion of 0-1.4 g kg^{-1} of dry matter ImmuPlus® in the diets of lambs, for 35 days.

Results: The inclusion of the polyherbal mixture ($P<0.01$) improved average daily gain (ADG), final live weight (FBW), feed conversion (FC), dry matter (DM) intake; however, the said mixture did not modify the back-fat thickness or chop area ($P>0.05$).

Study Limitations/Implications: The large number of plants and bioactive compounds included in ImmuPlus® limits the identification of the components that modify the metabolism of lambs.

Findings/Conclusions: The inclusion of 1.4 g kg^{-1} dry matter of ImmuPlus® in the diet of lambs improved their productive performance.

Keywords: additive, alternative ingredients, productive performance, sheep.

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INTRODUCTION

There is an overall increase in the value of herbal products, as a result of their bioactive compound content (*e.g.*, saponins, tannins, essential oils, and flavonoids) that can positively modify ruminal fermentation, volatile fatty acids (VFAs), and methane production in the rumen (Patra and Saxena, 2010).

A wide variety of herbal products that provide benefits to livestock production is available in the market; however, many products do not include reliable information or

have not been validated. Consequently, the evaluation and validation of these products are fundamental. ImmuPlus® is a polyherbal feed additive (PFA) mixture, prepared with a combination of Indian plants, that include a concentration of antioxidants, polyphenols, and flavonoids (Khan *et al.*, 2020). The inclusion of ImmuPlus® in the diets of rabbits improved their productive performance (Pulido, 2018). Regarding bovines, it benefited their ruminal fermentation (Ponce, 2018; Razo-Ortiz *et al.*, 2020) and health (Das *et al.*, 2003). Finally, it improved the weight gain of goats (Roy *et al.*, 2003) and the IgG serum levels of dairy cattle that suffer from mastitis (Mukherjee *et al.*, 2010).

Supplementing the diets of fattening lambs with ImmuPlus® is an excellent option for the improvement of weight gain and feed conversion; additionally, it modifies the ruminal environment. Nevertheless, ImmuPlus® should be added in a 1 to 1.4 g kg^{-1} DM range (Razo-Ortiz *et al.*, 2020; Dorantes-Iturbide *et al.*, 2022), because higher doses can impact productive performance.

The benefits of the bioactive compounds of ImmuPlus® used to supplement livestock diets is an alternative for the improvement of the productive performance of fattening lambs. Therefore, the objective of this experiment was to verify and validate previous researches about the effect of the inclusion of different doses of ImmuPlus® in the productive performance and the characteristics of the carcass variables of fattening lambs.

MATERIALS AND METHODS

The experimental procedures complied with the ethical, biosecurity, and animal welfare standards of the Colegio de Postgraduados and the Mexican official standard NOM-062-ZOO-1999 (use of animals in experiments). The experiment took place in the Colegio de Postgraduados, Campus Montecillo, State of Mexico, located at 98° 48' 27" W and 19° 48' 23" N, at 2,241 m.a.s.l. The area has a 15.9 °C annual mean temperature.

A completely randomized design was used to distribute 40 male lambs (Hampshire×Suffolk), with a 33.6±1.9 kg of initial live weight. Table 1 shows that the treatments consisted of a dietary concentration of the ImmuPlus® (Nuproxa México, Querétaro, Mexico) polyherbal phytogenic: 0-1.4 g kg^{-1} DM were added to a basal diet that sought to obtain a 300 g daily weight gain (NRC, 2007). The lambs were housed in individual metabolic cages, with individual feeders and nipple drinkers. Before the experiment took place, the lambs were orally dewormed (5% Closantil®, 20 mg kg^{-1} LW) and were intramuscularly vaccinated against *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordelli*, *Clostridium perfringes*, *Pasteurella multocida* type A, *Pasteurella multocida* type D, and *Pasteurella haemolytica* (Bobact® 8, 2.0 Ml $lamb^{-1}$). The feed was provided at 08:00 and 15:00 h, to guarantee a 5-15% rejection. The lambs had *ad libitum* access to water and feed. The animals were subjected to a 7-day adaptation period to the experimental diets. The experiment lasted 35 days.

The following variables were evaluated: daily feed intake (DFI), average daily gain (ADG), feed conversion (FC), and final live weight (FBLW). The back-fat thickness and *longissimus dorsi* (chop) area were measured between the 12^{th} and 13^{th} ribs at day 35, using a SonoVet 600 real-time ultrasound (Medison Inc., Cypress, CA, USA), with a 7.5 Mhz transducer.

Table 1. Nutritional and ingredient composition of the experimental diets.

Ingredient (g kg^{-1})	Control	Immuplus
Corn	210.0	210.0
Sorghum	322.2	334.5
Soybean meal	116.8	117.1
Alfalfa hay	156.2	154.3
Oat staw	143.8	131.7
Cane molasses	40.0	40.0
Immuplus[†]	0.00	1.4
Common salt	1.0	1.0
Premix[¶]	10.0	10.0
Calculated nutritional composition (Base to DM)[§]		
Metabolizable energy (Mcal kg^{-1})	2.8	2.8
Crude protein (g kg^{-1})	147.0	147.0
Detergent acid fiber (g kg^{-1})	192.0	187.0
Calcium (g kg^{-1})	7.0	7.0
Phosphorus (g kg^{-1})	3.4	3.4

[†]Immuplus®, Nuproxa Mexico, Querétaro, Mexico. [¶]Composition of the pre-mixture per kg: 100 g phosphorous, 120 g calcium, 5 g iron, 1 g magnesium, 1.5 g copper, 1.2 g zinc, 0.5 g manganese, 0.5 g cobalt, 0.2 g iodine, 0.2 mg selenium, 50,000 IU vitamin A. [§] Values calculated following the recommendation of NRC (2007).

Statistical analysis

The Shapiro-Wilk and Levene tests were used to test the normality distribution and the homogeneity of variance of the variables. The data were analyzed with the GLM procedure of SAS 8.0 (2010, Statistical Analysis System Inc. Cary, NC, USA). Tukey's test was use to compare the means of the treatments ($P \leq 0.05$) resulting from the different levels of Immuplus®. The initial live weight was the covariable for weight gain and final weight.

RESULTS

Table 2 shows that the inclusion of 1.4 g kg^{-1} dry matter of ImmuPlus® in the diet improved final live weight, average daily gain, feed intake, and feed conversion ($P > 0.05$). However, the addition of ImmuPlus® did not modify ($P > 0.05$) the dorsal fat thickness and the chop area (Table 2).

DISCUSSION

Polyherbal mixtures usually include several molecules with one or more active substances or predominant nutrients, responsible for their biological effects (Frankič *et al*., 2009). ImmuPlus® includes *Tinospora cordifolia*, *Ocimum sanctum*, *Whitania somnifera*, *Andrographis paniculata*, and *Azadirachta indica*, with a concentration of antioxidants, polyphenols, and flavonoids (Khan *et al*., 2020).

Table 2. Dietary addition of Immuplus® on the productive performance of lambs (35 d).

	Immuplus (g kg^{-1} of DM)		SE	P
	0	1.4		
ILW, kg	33.25	33.96	0.60	-
FLW, kg	43.55^{b}	48.02^{a}	0.66	0.0002
ADG, kg	0.28^{b}	0.41^{a}	0.02	0.0002
Feed intake, kg	1.58^{b}	1.74^{a}	0.03	0.0008
Feed conversion	5.78^{a}	4.29^{b}	0.28	0.002
Dorsal fat thickness, mm	3.29	3.10	0.13	0.35
Chop area, mm^2	1118.70	1085.19	31.69	0.47

SE: standard error of the mean; ILW: Initial live weight; FLW: final live weight; ADG: average daily gain; DM: Dry matter. ^{a,b}Means with different literals are statistically different ($P \leq 0.001$).

O. sanctum has essential oils with antimicrobial properties, such as camphor, eugenol, and eucalyptol (Yamani *et al.*, 2016). *A. paniculata* has flavonoids and essential oils with immunomodulatory and anti-inflammatory properties (Hossain *et al.*, 2021; Kumar *et al.*, 2021). In addition, it modifies the ruminal metabolism of goats, improving nutrient digestibility (Yusuf *et al.*, 2017). *A. indica* has tannins, flavonoids, and essential oils with anti-inflammatory and antioxidant properties (Sarkar *et al.*, 2021) and it can be potentially used to feed lambs (50 mg kg^{-1} feed) (Webb *et al.*, 2022). In addition, it interacts with the rumen microbes and influences the ruminal fermentation pattern, inhibiting methane production and increasing feed digestibility (Akanmu *et al.*, 2020). *W. somnifera* has flavonoids, tannins, and alkaloids with antimicrobial and antifungal properties. This plant can modulate the immune response in chicks (Latheef *et al.*, 2013) and it is used as anthelmintic on sheep (Miaron *et al.*, 2005). *T. cordifolia* has alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins (Upadhyay *et al.*, 2010). It modulates the immune response in chicks (Latheef *et al.*, 2013) and cows (Mukherjee *et al.*, 2010; Mallick and Prakash, 2011; Gupta *et al.*, 2016). In addition, it increases the antioxidant capacity and the cholesterol concentration in the semen (1 g kg^{-1} live weight) of rams, protecting the spermatozoa during cryopreservation (Jayaganthan *et al.*, 2013). Finally, it raises the antioxidant profiles in rats (Rawal *et al.*, 2004), helping to preserve membrane transportation and the fertilization capacity of the spermatozoa.

Supplementing the diets with some polyphenols acts as an antimicrobial agent against bacteria, protozoa, and fungi. Depending on the content (Huang *et al.*, 2018; Kalantar, 2018), type, and structure of the polyphenol, the said supplement can be beneficial or cause damage to feed consumption, nutrient digestibility, and growth (Waghorn, 2008).

The consumption of flavonoids in the diets of ruminants can potentially suppress methane production (Kalantar, 2018). Alhidary and Abdelrahman (2016) and Muqier *et al.* (2017) have proven that flavonoid supplementation increases weight gain and feed consumption in sheep. The flavonoid of some plants improves the immune response and the activity of antioxidant enzymes in blood serum (Hao *et al.*, 2020) and promotes the protein synthesis in sheep (Qin *et al.*, 2020).

Tannins can improve animal productivity (Huang *et al.*, 2018) and protein digestion. They also mitigate gas emissions produced during ruminal fermentation (Naumann *et al.*, 2017) and carry out antimicrobial and antiparasitic activities (Huang *et al.*, 2015; Malik *et al.*, 2017; Cipriano-Salazar *et al.*, 2018). Tannin supplementation improves protein digestibility, reduces energy loss resulting from the methane emission reduction, and increases the metabolic antioxidant capacity of lambs (Orzuna-Orzuna *et al.*, 2021a).

Essential oils improve the composition and functioning of the ruminal microbiota, the development of ruminal papillae in meat livestock (Zhang *et al.*, 2021), and the immune response (Wu *et al.*, 2021); in addition, they increase the VFA of the rumen (Zhang *et al.*, 2021).

Das *et al.* (2006) pointed out that ImmuPlus® is a non-toxic product that can be included in the diet of non-ruminant species, benefiting the animals. ImmuPlus® improved weight gain, feed consumption, and carcass yield in rabbits (Pulido, 2018). It improved ruminal fermentation and reduced the *in vitro* methane production of dairy cattle and sheep (Ponce, 2018; Razo-Ortiz *et al.*, 2020). In addition, ImmuPlus® increased the lgG levels in cows, helping to prevent and treat clinical mastitis (Das *et al.*, 2003). Finally, it has been used as antiparasitic in the diets of goats, improving their weight gain (Roy *et al.*, 2003).

Dorantes-Iturbide *et al.* (2022) pointed out that providing a low dose of ImmuPlus® (1 g kg^{-1} DM) to fattening lambs could promote feed conversion and increase the *longissimus* muscle area, consequently increasing their growth in the finishing stage; different results were obtained with 0, 2, and 3 g kg^{-1} DM of ImmuPlus®. Therefore, a low dose of ImmuPlus® can improve the productive performance of finishing lambs; however, high concentrations of this product reduce growth rate. In addition, the ImmuPlus® supplementation did not impact DM consumption, carcass yield, chemical composition of the meat, and health of lambs. The lack of changes in the consumption and the immune system variables suggest that the PFA did not have an impact on the welfare of the lambs.

Razo-Ortiz *et al.* (2020) evaluated different concentrations of ImmuPlus® and reported that a low dose of the product (1.4 g kg^{-1} DM) improved DWG and feed conversion, modified the ruminal environment (through pH reduction), and increased the total production of VFA and butyrate. In addition, it potentially altered the immune response of lambs. The quadratic response of the productive variables of lambs supplemented with ImmuPlus® (1, 2, and 3 g kg^{-1} DM) showed that the metabolites in the polyherbal mixture have a negative effect with >1.4 g/kg DM doses; nevertheless, the toxicology evaluations indicate they are safe for consumption (Das *et al.*, 2006). This hormetic response can be explained by the metabolites included in the PFA (Razo-Ortiz *et al.*, 2020).

CONCLUSIONS

The inclusion of ImmuPlus® improves the productive performance and the feed efficiency of fattening lambs.

REFERENCES

Alhidary, I.A., & Abdelrahman, M.M. (2016). Effects of naringin supplementation on productive performance, antioxidant status and immune response in heat-stressed lambs. *Small Ruminant Research*, 138, 31-36.

Akanmu, A.M., Hassen, A., & Adejoro, F.A. (2020). Gas production, digestibility and efficacy of stored or fresh plant extracts to reduce methane production on different substrates. *Animals, 10*(1), 146.

Cipriano-Salazar, M., Rojas-Hernández, S., Olivares-Pérez, J., Jiménez-Guillén, R., Cruz-Lagunas, B., Camacho-Díaz, L.M., & Ugbogu, A. E. (2018). Antibacterial activities of tannic acid against isolated ruminal bacteria from sheep. *Microbial pathogenesis*, 117, 255-258.

Das, P.K., Das, M.R., Acharya, K.C., & Ray, S.K. (2003). Evaluation of herbal immuno-stimulant" Immu-21" in prevention and treatment of bovine clinical mastitis. *Phytomedica*, 4, 13-20.

Das, S.N., Sharma, A., Mandil, R., & Agrawala, S.K. (2006). Sub-acute toxicity study of herbal E-50. *Phytomedica*, 7, 63-73.

Dorantes-Iturbide, G., Orzuna-Orzuna, J.F., Lara-Bueno, A., Miranda-Romero, L.A., Mendoza-Martínez, G.D., & Hernández-García, P.A. (2022). Effects of a polyherbal dietary additive on performance, dietary energetics, carcass traits, and blood metabolites of finishing lambs. *Metabolites, 12*(5), 413.

Frankič, T., Voljč, M., Salobir, J., & Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta Argiculturae Slovenica*, 94, 95-102.

Gupta, A.K., Sannat, C., Agrawal, R., & Hirpurkar, S.D. (2016). Effect of feeding of *Tinospora cordifolia* on immune response in cattle. *Journal of Animal Research, 6*(4), 579-584.

Hao, X., Wang, P., Ren, Y., Liu, G., Zhang, J., Leury, B., & Zhang, C. (2020). Effects of *Astragalus membranaceus* roots supplementation on growth performance, serum antioxidant and immune response in finishing lambs. *Asian-Australasian Journal of Animal Sciences, 33*(6), 965-972.

Hossain, S., Urbi, Z., Karuniawati, H., Mohiuddin, R.B., Moh Qrimida, A., Allzrag, A.M.M., Ming, L.C., Pagano, E., & Capasso, R. (2021). *Andrographis paniculata* (burm. F.) wall. Ex nees: an updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. *Life, 11*(4), 348.

Huang, Q. Q., Jin, L., Xu, Z., Barbieri, L. R., Acharya, S., Hu, T. M., McAllister, T. A., Stanford, K., & Wang, Y. (2015). Effects of purple prairie clover (*Dalea purpurea* Vent.) on feed intake, nutrient digestibility and faecal shedding of *Escherichia coli* O157: H7 in lambs. *Animal Feed Science and Technology*, 207, 51-61.

Huang, Q., Liu, X., Zhao, G., Hu, T., & Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal. *Animal Nutrition, 4*(2), 137-150.

Jayaganthan, P., Perumal, P., Balamurugan, T.C., Verma, R.P., Singh, L. P., Pattanaik, A.K., & Kataria, M. (2013). Effects of *Tinospora cordifolia* supplementation on semen quality and hormonal profile in rams. *Animal Reproduction Science, 140*(1-2), 47-53.

Kalantar M. (2018). The importance of flavonoids in ruminant nutrition. *Archives of Animal Husbandry & Dairy Science, 1*(1), 1-4.

Khan, T. A., Ipshita, A. H., Mazumdar, R. M., Abdullah, A. T. M., Islam, G. M. R., & Rahman, M. M. (2020). Bioactive polyphenol profiling and *in-vitro* antioxidant activity of *Tinospora cordifolia* Miers ex Hook F and Thoms: A potential ingredient for functional food development. *Bangladesh Journal of Scientific and Industrial Research, 55*(1), 23-34.

Kumar, S., Singh, B., & Bajpai, V. (2021). Andrographis paniculata (Burm. f.) Nees: Traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance. *Journal of Ethnopharmacology*, 275, 114054.

Latheef, S.K., Dhama, K., Wani, M.Y., Samad, H.A., Barathidasan, R., Tiwari, R., & Rai, R.B. (2013). Ameliorative effects of four herbs (*Withania somnifera*, *Tinospora cordifolia*, *Azadirachta indica* and E care SE herbal) on the pathogenesis of chicken infectious anaemia virus. *Internacional Journal of Current Research*, 5, 2327-2331.

Malik, P. K., Kolte, A. P., Baruah, L., Saravanan, M., Bakshi, B., & Bhatta, R. (2017). Enteric methane mitigation in sheep through leaves of selected tanniniferous tropical tree species. *Livestock Science*, 200, 29-34.

Mallick, S., & Prakash, B.S. (2011). Effects of supplementation of *Tinospora cordifolia* to crossbred cows peripartum. *Animal Reproduction Science, 123*(1-2), 5-13.

Miaron, J.O., Wahome, P.G., & Mapenay, I.M. (2005). Anthelminthic activity of *Withania somnifera*. L. Dunal water extract in sheep. *East and Central African Journal of Pharmaceutical Sciences, 8*(1), 6-9.

Mukherjee, R., De, U.K., & Ram, G.C. (2010). Evaluation of mammary gland immunity and therapeutic potential of *Tinospora cordifolia* against bovine subclinical mastitis. *Tropical Animal Health and Production, 42*(4), 645-651.

Muqier, Q.S., Wang, T., Chen, R., Wang, C., & Ao, C. (2017). Effects of flavonoids from *Allium mongolicum* Regel on growth performance and growth-related hormones in meat sheep. *Animal Nutrition, 3*(1), 33-38.

Naumann, H.D., Tedeschi, L.O., Zeller, W.E., & Huntley, N.F. (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Revista Brasileira de Zootecnia*, 46, 929-949.

NRC, National Research Council. (2007). Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids. 7th Ed. National Academy Press. Washington, DC, USA. 384p.

Orzuna-Orzuna, J.F., Dorantes-Iturbide, G., Lara-Bueno, A., Mendoza-Martínez, G.D., Miranda-Romero, L.A., & Hernández-García, P.A. (2021). Effects of dietary tannins' supplementation on growth performance, rumen fermentation, and enteric methane emissions in beef cattle: a meta-analysis. *Sustainability,13*(13), 7410.

Patra, A.K., & Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry* 71, 1198-1222.

Ponce, P.O. (2018). Efecto de la adición de una formula poliherbal (Immuplus) sobre los parámetros productivos y de salud de becerras. Tesis de Licenciatura. Universidad Autónoma del Estado de México.

Pulido H.S. (2018). Efecto de la adición de fórmula poliherbal sobre respuesta productiva, calidad y oxidación lipídica de carne de conejo. Tesis de Maestría. Universidad Autónoma del Estado de México.

Qin, X., Zhang, T., Cao, Y., Deng, B., Zhang, J., & Zhao, J. (2020). Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. *Meat Science*,166, 108141.

Rawal, A.K., Muddeshwar, M.G., & Biswas, S.K. (2004). *Rubia cordifolia*, *Fagonia cretica* linn and *Tinospora cordifolia* exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. *BMC Complementary and Alternative Medicine, 4*(1), 1-9.

Razo-Ortiz, P.B., Mendoza-Martinéz, G.D., Silva, G.V., Osorio-Teran, A.I., González-Sánchez, J.F., Hernández-García, P.A., de la Torre-Hérnandez M.E., & Espinosa-Ayala, E. (2020). Polyherbal feed additive for lambs: effects on performance, blood biochemistry and biometry. *Journal of Applied Animal Research, 48*(1), 419-424.

Roy, S., Tiwari, A., & Roy, M. (2003). Studies on the efficacy of herbal Zycox alone and in combination with ImmuPlus in caprine coccidiosis. *Phytomedica*, 4, 29-33.

Sarkar, S., Singh, R.P., & Bhattacharya, G. (2021). Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *Biotech*, 11, 178.

Upadhyay, A.K., Kumar, K., Kumar, A., & Mishra, H.S. (2010). *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)-validation of the Ayurvedic pharmacology through experiment and clinical studies. *International Journal of Ayurveda Research*, 1, 112-21.

Waghorn, G. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-progress and challenges. *Animal Feed Science and Technology,147*(1-3), 116-139.

Webb, E. C., Hassen, A., Olaniyi, M. O., & Pophiwa, P. (2022). Effect of Dietary Inclusion of *Azadirachta indica* and *Moringa oleifera* leaf extracts on the carcass quality and fatty acid composition of lambs fed high forage total mixed rations. *Animals, 12*(16), 2039.

Wu, J.P., Zhou, R., Liu, L.S., Casper, D.P., Lang, X., Wang, C.L., Zhang, L.P., Wei, S., & Liu, H.B. (2021). Growth performance, nutrient digestibility, blood parameters, and carcass characteristics by lambs fed an oregano and cobalt blend. *Animal*, 15, 100365.

Yamani, H.A., Pang, E.C., Mantri, N., & Deighton, M.A. (2016). Antimicrobial activity of tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. *Frontiers in Microbiology*, 7, 681.

Yusuf, A.L., Adeyemi, K.D., Samsudin, A.A., Goh, Y.M., Alimon, A.R., & Sazili, A.Q. (2017). Effects of dietary supplementation of leaves and whole plant of *Andrographis paniculata* on rumen fermentation, fatty acid composition and microbiota in goats. *BMC Veterinary Research, 13*(1), 1-9.

Zhang, R., Wu, J., Lei, Y., Bai, Y., Jia, L., Li, Z., Liu, T., Xu, Y., Sun, J., Wang, Y., Zhang, K., & Lei, Z. (2021). Oregano essential oils promote rumen digestive ability by modulating epithelial development and microbiota composition in beef cattle. *Frontiers in nutrition*, 8, 722557.