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ARTICLE

Parasite Fauna of *Lutjanus synagris* Commercialized in the Fish Market from Bragança-PA, Brazil

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ABSTRACT

Studies about the parasite fauna of marine fish highlights as an important problem for public health with zoonotic parasites or affecting the fish quality. Thus, this study evaluated the parasite fauna of *Lutjanus synagris* commercialized in the fish market from Bragança-PA. In laboratory, 58 fish were measured, weighted and conducted to parasitological analysis to determine parasitological indexes and relative dominance. Every parasite was fixed and identified until to the lowest taxonomic level. Through the parasitological analysis, it found Cymothoidae, Digenea, *Cucullanus* sp. and *Procamallanus (Spirocamallanus)* sp., with total prevalence 67.24%. Digenea showed the highest prevalence and mean intensity values. For nematode, *Cucullanus* sp. obtained the greater prevalence and relative dominance, while *Procamallanus (Spirocamallanus)* sp. showed the greater mean intensity and abundance. *Cymothoidae* showed the lowest prevalence and mean intensity values. As conclusion, the parasite fauna of *L. synagris* has been noted with low diversity, reporting the nematode occurrence *Procamallanus (Spirocamallanus)*.

1. Introduction

Fishing activity, an important source of fish meat for human consumption, generated approximately 91 million of tons at 2016 [1,2]. Fish species from Lutjanus family are distributed in the tropical and subtropical regions with carnivorous habit and high commercial value, widely appreciated by consumers [3,4]. Among the fish species, ariacó *Lutjanus synagris* commonly known as red fish are appreciated for all northeast region from Pará due to the meat quality and price.

Despite the commercial importance of this species for human consumption, its trade can represents risk to public health with zoonotic parasites [5,6,7]. In addition, the high

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parasite infestation can provoke physiological alterations in the fish, making it more susceptible to diseases [8,9].

Currently, still are few scientific papers about the parasite fauna for genus Lutjanus, with reports of crustaceans, monogenea, nematode and cestode [5,7,10,11,12]. In front of this, take knowledge about the parasite fauna can contributes with important information of national marine parasites. Thus, this study evaluated the parasite fauna of *L. synagris* commercialized in the fish market from Bragança PA.

2. Material and Methods

Species of *L. synagris* were purchased between September and October at 2018 of fish market from Bragança PA (1º05'42.94" S and 47º16'19.52" W) adequately packed in plastic bags and conducted to laboratory. Every fish were identified according to Lessa and Nóbrega [13], measured (TL: total length) and weighted (W: weight).

Parasitological analysis were carried out according to Eiras *et al*. [14] and Amato *et al*. [15], being quantified, collected, fixed and identified until the lowest taxonomic level[16]. Based on parasite quantification, this study determined the parasitological indexes as prevalence (P), mean intensity (MI), mean abundance (MA) [17] as well as the relative dominance (RD) [18].

3. Result and Discussion

About the 67.24% of analyzed *L. synagris* (58 fish 26.55 ± 5.12 cm 482.12 ± 186.30 g) has been infested by parasite at least one taxon. This study identified four taxon (table 1). The scientific literature has been reported crustaceans (*Lernantrhopus* sp.) nematode (*Anisakis* sp., *Capillaria* sp. e *Cucullanus* sp.) and cestode (Larvas de *Floriceps* sp.) for *L. synagris* [5,7,10,19,20].

Table 1. Parasitological indexes, relative dominance and infestation site on *L. synagris* commercialized in the fish market from Bragança PA

Parasite	IS	P (%)	MI	MA	RD
Cymothoidae	G/M	10.34	1.33	0.14	0.04
Digenea	PS/I	62.07	3.44	2.14	0.59
Cucullanus sp.	I	46.55	1.93	0.90	0.25
***Procamallanus (Spirocamallanus)* sp.**	I	13.79	3.38	0.47	0.13

Notes: IS - Infestation site, P – prevalence, MI – mean intensity, MA – mean abundance, RD – relative dominance, G/M – gill/mouth, PS/I – pyloric cecum, I – intestine.

Digenea, found in the pyloric cecum and intestine, showed the greater prevalence, mean intensity, mean abundance and relative dominance (table 1). Within found nematode, *Cucullunus* sp. obtained the greater prevalence (46.55%) and relative dominance (0.25), while *Procamallanus* (*Spirocamallanus*) sp. showed greater mean intensity (3.38) and mean abundance (0.47).

Fish are considered intermediary host to the digenea life cycle [21], being a common parasite into aquatic ecosystem widely reported to L. *guttatus; L. adetti* and *L. fulviflamma* [22,23,24]. According to the Argáez-García *et al*. [19], they found digenea species *Hamacreadium mutabile*, *Helicometrina nimia*, *Metadena globosa*, *Stephanosthomum casum*, *Paracryptogonimus americanos*, *Hemiurus sp*. and *Neoprosorhynchus* in the pyloric cecum, intestine and stomach of *L. griseus*.

According to the Morales-Serna *et al*. [23], evaluating the parasite fauna of *L. guttatus* between 2004 and 2006, they found prevalence values of 0 to 21% for digenea, lower value if compared to the present study. However, its mean intensity for the same parasite (digenea) showed greater values (4.5) than this study.

The most studies of nematode at marine fish only describes the parasite [25,26,27]. The greater importance about this parasite would be its zoonotic potential [28,29]. In the present study, found nematodes have no zootechnical potential, different result if compared to the Alves *et al*. [7] with genus *Anisakis* sp. and *Raphidascaris* sp. (Ichthyascaris) at prevalence 17.39 and 4.34% respectively for *L. synagris*. Other study on the same fish species, they found cestode larvae (*Floriceps* sp., *Pseudogrillotia* sp. *Oncomegas* sp.) with the first report about *Philometrai* sp. [20,25], a parasite which affects the fish meat.

According to González-Solís *et al*. [30], they identified nematode *Cucullanus* in fish species *Arothron hispidus, Abudefduf sordidus* and *Caranx ignobilis* with prevalence 47% and mean intensity (6±4.7) for *A. hispidus* species. The prevalence results were similar to the present study, this being the nematode group with greater relative dominance (0.25). None study is related to the presence of the genus Procamallanus (Spirocamallanus) in *L. synagris*, which is probably caused by the consumption of zooplankton, which is considered a parasite in the egg or larvae phase [31].

In the present study, despite the low prevalence (10.34%), mean intensity (1.33), medium abundance (0.14) and relative dominance (0.04) observed for the crust of the Cymothoidae family, this record is relevant to survey the parasitic fauna of *L. synagris*. The reports by Cavalcante *et al*. [10] observed this same species of fish the parasites *Lernantrhopus* sp., *Lernaelophus striatus* and *Rocinela* sp. that were found in the gills and mouth. Therefore, information about a parasitological fauna of marine species with economic value in the market is essential for the management of the commercialization of this fish.

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4. Conclusion

The *Lutjanus synagris* has been noted with a low parasite fauna, with the greater prevalence for digenea and occurrence of nematode *Procamallanus* (*Spirocamallanus*) sp.

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REVIEW

The Importance of Motacilla Alba Behavior on Hitting Its Own Mirror Reflection

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ABSTRACT

Self-awareness is considered as a capability of recognize oneself and increasingly received attention. However, self-awareness in the bird Motacilla Alba is unclear. To study the self-recognition in Motacilla Alba, the subject is observed by mirror while eating. The bird performed the look around, confirm again the surroundings, become alert, hit the mirror. These behaviors suggests that presently Motacilla Alba does not have the capacity of self-awareness by the test.

1. Self-awareness Process

The ability to recognize oneself in the mirror is considered as self-awareness [1]. Mirror self-recognition (MSR) is designed as a method to explore the animal's sense of self [2]. Tests of mirror self-recognition (MSR) have been central to our understanding of self-awareness from developmental and evolutionary perspectives.

Self-awareness in humans spontaneously emerged [3]. In 2000,Kusayama et al showed that four jungle crows (Corvus macrorhynchos) were exposed to a mirror with peck and flap behaviors but species failed to pass the MSR [4]. In 2002, Watanabe experimented that java sparrows were equaled to choose a mirror and a frosted live bird when they were exposed to a mirror and a frosted mirror. The result suggested that java sparrows saw the self-image on the mirror as conspecific image [5]. Furthermore, In 2017, Fanny-Linn Kraft tested the great tit mirror response with social behavior. The result showed no evidence that the great tit possessed self-awareness [6].

2. Fly to Mirror Behavior

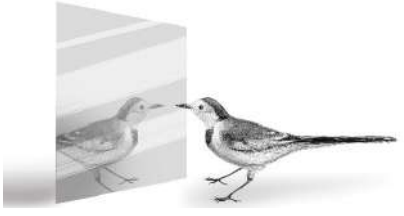

Figure 1. Check the subject in the mirror

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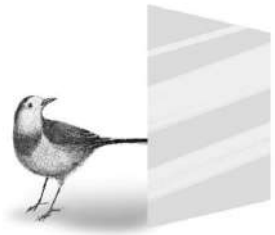

Figure 2. Check who/which hide behind the mirror

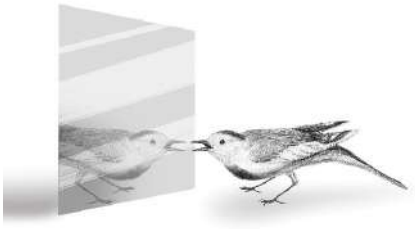

Figure 3. Get angry about the unknown subject

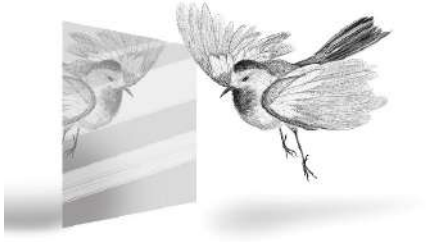

Figure 4. Fly to Mirror Behavior

Interestingly, we have set up a mirror on the ground in the open field. The bread was placed in front of the mirror. In this test, we found that Motacilla alba looked around before it approached the bread, see Figure 1. The reflection of mirror was found by the subject while picking food. It became more nervous. Then it walked to the back of the mirror to confirm whether there is conspecifics behind of the mirror for three times, see Figure 2, 3. At the beginning, the subject picked the food peacefully. It became more and more alert when it saw the reflection in the mirror. Ultimately, Motacilla alba violently attacked the reflection continuously in the mirror and a bright chirp, see Figure 4.

The MSR is an excellent method to test self-awareness. Motacilla alba presented the aggressive behavior when saw its reflection in the mirror. Unfortunately, neither of these behaviors could be demonstrate that the Motacilla alba can recognized itself in the mirror.

3. Meanings

Figure 5. Aggressive behavior-Fly to mirror

So far, most of the species on the MSR tested have failed to identify itself in the mirror. Many of them responded to their self-image with social behavior if considered the image their conspecifics. Some species have shown aggressive behavior [7]. Even humans aren't born with a sense of self. We may not recognize ourselves in a mirror from 15 to 24 months of age [8]. There is an important evidence that only a few species can pass the MSR test, and only long-term use of mirrors as visual stimuli can pass the MSR test such as (four great apes [9], bottlenose dolphins [10]. Asian elephants [11], and magpies [12]. Some species have the possibility to modify their self-characteristics through learning [13]. This proves to a certain extent that self-consciousness requires certain conditions, which can be learned. However it cannot be absolutely denied that those animals (fishes, birds, sea lions, dogs and cats [14]) have no sense of self. It only proves that they are not self-aware at this stage. When the birds are facing the similar selection pressures that leads to changes in neural structure, particularly in cognition [15]. Our data demonstrated that Motacilla alba are not capable to recognize itself in the mirror for the first time.

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ARTICLE

Milk Fatty-Acid Profile after Feeding Increasing Doses of a Mixture of Soybean and Linseed Oils to Pasture Dairy Cows

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ABSTRACT

The goal was to determine the effect of growing intake of a mixture (75:25) of soybean (SoOi) and linseed (LiOi) oils on milk production and composition and milk fatty-acid (MF-A) profile in grazing dairy cows. Twenty-four Holstein cows were assigned to 4 treatments in a completely randomized design with three weeks of adaptation to oil doses and one week of experimental measurements. On a dry matter (DM) basis, cows were fed pasture (63%), energy concentrate (37%) and the SoOi LiOi oil mixture at zero (Tr0%), 2% (Tr2%), 4% (Tr4%) and 6% (Tr6%) of total DM intake equivalent to 0, 0.36, 0.72 and 1.08 kg/cow/day of the oil mixture. The oil mixture was manually mixed-up to the concentrate (7.04 kg DM/cow/day) and supplied by halves during each milking time without refusals. Pasture ($P = 0.49$) and total DM intakes ($P = 0.31$) were similar between treatments averaging 11.27 and 18.85 kg DM/cow/day respectively. Milk output (22.71 kg/cow/day) was not affected ($P = 0.46$). Milk fat content reduced linearly ($P < 0.05$) from 3.20 (Tr0%) to 2.67 g/100g (Tr6%) without effects ($P = 0.73$) on fat or fat corrected milk (4%FCM) yields. Milk protein concentration ($P < 0.56$) or yields ($P < 0.11$) were not affected. Lactose contents tended ($P < 0.08$) to be higher in oil supplemented cows and milk urea nitrogen was not affected ($P = 0.14$). The basal (Tr0%) concentration (g/100g MF-A) of totaly hypercholesterolemic MF-A ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) averaged 38.93 and decreased linearly ($P < 0.0001$) with oil intake to 37.81 (Tr2%), 31.59 (Tr4%) and 29.18 (Tr6%). Levels of elaidic (*trans*-9 $C_{18:1}$) and *trans*-10 $C_{18:1}$ MF-A resulted low-slung in the basal (Tr0%) milk (0.21 and 0.20 g/100g MF-A, respectively) but increased linearly ($P < 0.0001$) after oil intake reaching the maximum values at Tr6% (0.73 and 2.23 g/100g MF-A, respectively). Milk concentration (g/100g MF-A) of vaccenic acid (*trans*-11 $C_{18:1}$, VA) averaged 3.63 in Tr0% and increased linearly ($P < 0.0001$) with oil intake reaching 4.97, 7.05 and 8.38 in Tr2%, Tr4% and Tr6%, respectively. Basal concentration of rumenic acid (*cis*-9. *trans*-11 $C_{18:2}$, RA) was 2.28 g/100g MF-A and increased linearly ($P < 0.0001$) with increased oil dose resulting in maximal plateau in Tr4% (3.88) and Tr6% (3.89). The basal atherogenic index (AI) of milk was 1.87 and linearly decreased ($P < 0.01$) to 1.64 (Tr2%), 1.18 (Tr4%) and 0.95 (Tr6%) after oil intake. The basal Ω6/Ω3 ratio (3.57) was no different ($P > 0.05$) from Tr2% (3.37) but was upper ($P < 0.05$) in Tr4% (4.41) and Tr6% (4.63) remaining under the recommended value of 5:1. Taken together the results suggest that feeding a blend (75:25; SoOi) of SoOi an LiOi oils at 4% of total DM intake to grazing dairy cows maximize the milk RA content with a concomitant decrease in the hypercholesterolemic MF-A of milk maintaining a beneficial for health Ω6/Ω3 ratio with low levels of the detrimental *trans*-9 $C_{18:1}$ and *trans*-10 $C_{18:1}$.

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1. Introduction

Milk F-A composition is a determinant factor of its healthy properties due to the potential effects that certain specific MF-A have on human health. In human diets, dairy fat can account up to 75% of total consumption of fat from ruminant origin and although dairy products (which have a very low cholesterol content) provide only 15-25% of the total fat, they provide about 25 to 35% of total milk saturated fat (MSF) consumed daily [1].

Some saturated FA present in milk just like lauric ($C_{12:0}$). myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) are potentially atherogenic when consumed in excess [2,3] and related with the increased risk of cardiovascular diseases [2,4]. Feeding oils high in polyunsaturated PUFA is an effective and natural tool to inhibit *de novo* mammary synthesis of milk saturated FA (MSF-A) reducing the presence of the pro-atherogenic MF-A of milk fat [5,6].

A special interest has been placed in RA, the *cis*-9. *trans*-11$C_{18:2}$ isomer of conjugated linoleic acid (CLA), for its potential healthy role on the levels and composition of circulating lipids, cardiovascular health [7,8] and the reduction in the incidence of some types of cancer [4,9,12] and immune response [13,14]. On the other hand, VA (*trans*-11 $C_{18:1}$) is the main trans MF-A being the most important precursor of RA [1]. It showed antiproliferative properties itself or after being converted to RA in human tissues at an estimated rate of 20% [15].

Dairy fat is the most important natural source of RA and its concentration in milk fat is highly dependent on type of diet and lipid supplementation [1,5,6,16]. A pasture-based diet allows to obtain a milk with a high basal level of RA which can be amplified feeding vegetable oils high in PUFA [1,5,6,17]. A mixture (75:25) of SoOi and LiOi showed to be very effective [6] but the optimal level of oil-blend supply has not yet been well defined. This is a subject of concern taking into account oils costs, deviations towards unhealthy *trans*-MF-A (*trans*-9 and *trans*-10 $C_{18:1}$) synthesis owing to oil overdoses and the potential deleterious effects of free oils on ruminal function and digestion. Despite the practical importance of knowing what is the most adequate quantity of oils to be supplied, in our knowledge experimental results are still very scarce or directly non-existent. A linear effect of oil intake on milk RA was postulated reaching a plateau at an oil dose of 4% of total DM intake [1]. The objective of the study was to define the most adequate quantity of the SoOi LiOi mixture (75:25) to be supplied to grazing dairy cows in order to obtain milk with up high level of CLA and reduced concentrations of unhealthy fatty acids at the lowest cost.

2. Materials and Methods

2.1 Treatments, Animals and Experimental Design

The experiment was carried out at the National Institute of Agricultural Technology (INTA) in Balcarce (37°45'S, 58°18'W) during September and October of 2014. The experimental period lasted 4 weeks (wk) with the first three wk as adaptation to oils intake and the fourth wk for data collection. Twenty-four multiparous Holstein cows (552 ± 50 kg, body weight, BW) in mid lactation (244 ± 69 days postpartum) and producing 20.5 ± 1.8 kg milk/day were allocated to four treatments (6 cows per treatment) in a completely randomized design. Treatments were defined by the increasing intake of a blend (w/w) of 75% (SoOi) and 25% (LiOi). The oil blend was consumed at 0% (Tr0%), 2% (Tr2%), 4% (Tr4%) and 6% (Tr6%) of total DM intake of the dairy cow (18 kg DM/cow/day) measured during the first wk prior to the start of the trial. Procedures and animal care were approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE, INTA CERBAS).

A perennial pasture of brome grasse (*Bromus unioloides*) and red clover (*Trifolium pratense*) was offered using a daily-strip grazing system. The area of each strip was regulated using a temporary electric fence to provide an herbage allowance (HA) of 27 kg DM/cow/day. The available total biomass (kg DM/ha) was estimated every week to adjust the size of the daily grazing strip by the double sampling method using the relationship between the height of the forage (x) and the available biomass (y) as described previously [18]. The equations were adjusted for both, initial availability and for the remaining forage after grazing. The concentrate included ground corn grain (35%), malt brewery waste (10%), pelletized sunflower meal (20%), soybean grains (10%), wheatgrass (21.48%), calcium carbonate (2%), magnesium oxide (0.4%), salt (1%), rumensin (0.02%) and a vitamin-mineral mix (0.1%). It was offered at a rate of 8 Kg/cow/day in two equal feedings during each milking time (06.00 and 16.00).

According to treatments, the daily dose of the oil-blend was manually mixed to the concentrate during each milking time and thoroughly consumed by cows. The effective quantities of the oil-blend consumed (kg/cow/day) were 0.36 (Tr2%), 0.72 (Tr4%) and 1.08 kg (Tr6%). Adaptation to oil intake proceeded gradually by feeding by halves the target daily dose at each milking time starting with 0.1 (Tr2%), 0.2 (Tr4%) and 0.3 (Tr6%) Kg/cow/day during the first day, 0.2, 0.3 and 0.4 Kg/cow/day for the next 2 days, 0.2, 0.3 and 0.5 Kg/cow/day at day 4 and full dose

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according to treatment from day 5 until the end of the trial.

The animals were milked twice a day at 6:00 a.m. and 4:00 p.m. and after each milking they were conduced to the pasture with fresh and clean water available *ad libitum*.

2.2 Sampling Measurements and Laboratory Procedures

Representative samples (0.5 kg) of pasture and concentrate were taken weekly. Pasture samples were collected from the grazing horizon by hand-plucking [19]. All samples were dried at 60°C for 48 hours in an oven with forced air circulation to determine DM content and then milled in a Willey mill (1 mm mesh). They were assayed for organic matter (OM) (muffle at 550-600°C for 4 hours), crude protein (CP) [20] with a LECO FP-528 analyzer), water soluble carbohydrates (WSC) [21], neutral (NDF) and acid (ADF) detergent fiber (using the filter bag technique [22] and [23] respectively) with an autoanalyzer (ANKOM Corp. Fairtport. New York. USA 1970). Ether extract (EE) was determined by the solvent extraction technique [24] with an autoanalyzer (ANKOM Corp. Fairtport. New York. USA). The *in vitro* DM digestibility (IVDMD) was estimated after 48 hours of incubation in a Daisy II ANKOM equipment. Starch content was determined as described in [25].

Pasture DM intake was individually estimated by the difference method [19] during 3 consecutive days in the week prior to the start of the experiment and during the last 3 days of week 4th. The average DM intake of the three consecutive days of measurements from each cow was computed for the statistical analysis.

Milk production was daily recorded over the whole experiment. Milk samples (50 ml) were collected at a.m. and p.m. milkings twice a week on non-consecutive days. The two samples were pooled according to the corresponding volume measured at each milking time and analyzed for fat, total protein, lactose, total and not-fat solids by mid-infrared spectrophotometry (Milko Scan-Minor, Foss Electric, Hillerod, Denmark). Milk urea nitrogen (MUN) was determined using a commercial enzymatic kit (Wiener Laboratories., Rosario, Argentina).

The cows were weighed on two consecutive days after the morning milking on days 6th 7th, 27th and 28th of the trial. Body weight (BW) change was calculated as the difference between the final minus the initial BW (average of two days) divided by the number of days elapsed. During the last two weeks of the trial, blood samples were taken by jugular vein puncture after the a.m. milking. Blood was collected in tubes containing EDTA (7-8 drops/tube, 0.342 mol/l, pH 7.2, Wiener Laboratory, Rosario. Argentina) and centrifuged (2000 × g for 15 min at 4°C). Plasma was collected and stored at –24°C until analysis for glucose, plasma urea nitrogen (PUN), triglycerides, and cholesterol using enzymatic kits (Wiener Laboratories, Rosario, Argentina). Non-esterified fatty acids (NEFA) were assayed using the enzymatic kit from Randox Laboratories Ltd (UK).

At day 21st of oil-blend supplementation and from each composite sample collected to determine the chemical composition of milk, aliquots of 50 ml were frozen (−24˚C) to obtain a single pool sample per cow for the determination of MF-A composition by gas liquid chromatography (GLC) as previously described [6]. Total milk fat (TMF) was determined gravimetrically by extraction with petroleum ether at 65-80°C [26]. The lipids were extracted with a mixture of hexane: isopropanol (3: 2) and 6% sodium sulfate at room temperature [27]. The lipid residue was dried at 40°C under a stream of nitrogen. For FA methyl esters (FAME) preparation, a cold method with hexane and 2N KOH in methanol was used [28]. The FAME were quantified using a gas chromatograph (GLC-Shimatzu GC-2014. Shimadzu Corporation. Kyoto. Japan) equipped with a CP-Sil 88 capillary column (100 mx 0.25 mm id., Varian. Lake Forrest. CA. USA) and a flame ionization detector. Injector and detector temperatures were maintained at 250°C, the flow rate at 1:100 and 1 µl of standard or milk sample using an automatic sampling device at each run of the GLC [29]. The hydrogen flow was fixed at 1 ml/min and the nitrogen flow (compression gas) at 25 ml/min. Maximum retention times and area percentages of total FA were identified by injecting known patterns. Internal standards [(Tritridecanoin [13: 0-triacylglycerol (TAG)], external reference standards GLC-463 (mixtures of 52 EMAG (purity> 99%) and *trans*-mix GLC 481 (purity> 99%) were purchased from Nu-Chek (Nu-Chek Prep. Inc., Elysian. MN. USA). Methyl esters of linoleic acid, *cis/trans* mixture (Catalog No. 47791), mixtures of unsaturated C_4-C_{24} chain length methyl esters (Catalog Nº 18919) and of the individual chain length FAMEs from $C_{4:0}$ to $C_{24:1}$ saturated and unsaturated were obtained from Supelco (Bellefonte. PA. USA). Mixtures of positional and geometric FA isomers were provided by the CYT-ED International Network (208RTR0343). The FAME were identified by comparing their retention times with commercial standards. The values were expressed as a percentage of the total FAME. The lower limit of quantification for the FAMEs identified varied from 0.01% to 0.03%. To convert g FAME/100g methys esthers to equivalents of triacilglycerides (TAG) (g of FA as TAG/100g of total TAG) the respective Conversion Factors tabulated in the AOCS Method Ce 1j-07 [29] were used. To estimate the

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g of MF-A 100g of sample, the g of MF-A 100g of TAG were multiplied by the total fat content (%). The results are expressed in g/100g of total MF-A.

2.3 Statistical Analyses

The effect of increased levels of the oil-blend intake on milk production and composition, MF-A profile and BW changes was analyzed by orthogonal contrasts taking into account the linear, quadratic and cubic effects using the PROCEDURE MIXED [30]. Results of DM intake and plasma metabolites were analyzed by PROCEDURE GLM [30] using the following model:

$$Y_{ij} = \mu + T_i + + E_{(i)j}$$

Where: Y_{ij} = the dependent variable, μ: overall mean, T_i = treatment effects and $E(ij)$ = the residual error associated with the *ij* observation. The threshold of statistical significance was stated at $P < 0.05$.

3. Results and Discussion

3.1 Forage and Oil Charasteristics

In the pre-grazing strips, pasture biomass averaged 2100 (± 308) kg DM/ha being above the critical value of 2000 kg DM/ha below which DM intake could be restricted [31]. The daily-strip grazing system used allowed to maintain the target HA of 27 (± 2) kg DM/cow/day adequate to obtain *ad libitum* pasture intake [31]. It has also been postulated that the maximum pasture DM intake would be achieved when HA ranges from 45 to 55 g DM/ kg BW/day [32]. For the average BW of cows (554 kg, Table 6), the HA range would be 25 to 30 kg DM/cow/day and therefore the 27 kg DM/cow/day obtained fell inside the proposed range. Pasture intake may also be affected if forage DM content is less than 18% following the linear relationship observed between pasture DM content and intake in the range of 13-22% DM content [33]. In our trial, pasture DM content averaged 20.5% (Table 1) therefore exceeding the critical range reported [33]. In the same way, pasture CP (24.1%) and NDF (36.4%) contents (Table 1) were found within the range of 15-25% (CP) and 36-54% (NDF) proposed by [31] to obtain a high forage digestibility as observed (70.93%) *in vitro* (Table 1). The WSC and EE contents may be considered normal for good quality pastures. It can be concluded that both, the quality and the amount of the pasture offered to cows were sufficient to achieve adequate DM and energy intakes. The chemical composition of the concéntrate (Table 1) was normal for a good quality energy concentrate.

Table 1. Chemical composition and *in vitro* dry matter (DM) digestibility of pasture and concentrate [(1)]

Parameter	Pasture[2]	Concentrate
Dry matter. %	20.50 ± 0.70	89.6 ± 0.65
Organic matter, % DM	91.66 ± 0.55	92.80 ± 0.46
Crude Protein, % DM	24.10 ± 1.56	17.32 ± 1.02
NDF, % DM	36.40 ± 1.00	23.97 ± 2.00
ADF, % DM	18.23 ± 1.11	11.51± 1.41
***In vitro* DM digestibility, %**	70.93 ± 0.40	75.14 ± 1.88
Starch, % DM	1.57± 0.40	32.59 ± 4.05
Ether Extract, % DM	3.23 ± 0.31	4.47 ± 0.77
Metabolizable Energy, Mcal/kg DM	2.89 ± 0.01	2.71 ± 0.07
Water Soluble Carbohydrates, % DM	12.00± 3.40	20.80± 1.51

Note: [1] Values expressed as the mean ± standard deviation. Pasture and concentrate (n = 4). [2] Consociated pasture containing Bromus unioloides and Trifolium pratense.

The MF-A composition of feedstuffs and oils used in the experiment is shown in Table 2.

Table 2. Fatty acids composition of feedstuffs and oils

Fatty acid	Pasture[1]	SoOi[2]	LiOi[3]	Concentrate
	g/100g FA			
$C_{16:0}$	14.08	10.67	6.71	13.91
$C_{18:0}$	1.31	4.31	5.38	1.92
***cis*-9 $C_{18:1}$**	1.53	17.43	18.28	26.89
***cis*-11 $C_{18:1}$**	0.18	2.01	3.16	4.13
***cis*-9 *cis*-12 $C_{18:2}$**	14.58	52.87	16.35	49.75
***cis*-9 *cis*-12 *cis*-15 $C_{18:3}$**	62.76	11.36	49.87	2.29

Note: [1]Consociated pasture containing Bromus unioloides and Trifolium pratense. [2] Soybean oil. [3] Linseed oil.

As expected, pasture and LiOi were rich in linolenic acid (*cis*-9 *cis*-12 *cis*-15 $C_{18:3}$). The average linoleic acid content of the pasture resulted higher than reported in other experiments [34,35] probably due to the high quality of the pasture used in the present trial. The SoOi was characterized by its high linoleic acid (*cis*-9 *cis*-12 $C_{18:2}$) content (52.87%) and by a low SFA concentration. Concentrate and oils were a good source of oleic acid (*cis*-9 $C_{18:1}$) as reported previously [36,39].

When a high quality pasture is included in cow's diet, rumen lipid metabolism is oriented to healthy changes in MF-A composition mainly concerning PUFA of the Ω3 series and CLA. A significant reduction of MSF-A-content and the increase of oleic acid are also expected [40]. Intake of high quality fresh pastures also prevents the shift and increase in concentration of unhealthy MF-A like *trans*-9 and *trans*-10 $C_{18:1}$ isomers.

3.2 Dry Matter Intake, Milk Yield and Composition

Feeding increased doses of the oil-blend mixed to the con-

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centrate did not affect ($P > 0.05$) concentrate, pasture or total DM intake of cows (Table 3).

Table 3. Intake of pasture, concentrate and oil in dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of total DM intake

Parameter	Treatment[1]				SEM	*P* - value[2]
Intake, kg/DM/cow	Control	Tr2%	Tr4%	Tr6%		
Pasture[3]	12.00	10.83	10.67	11.57	0.59	0.49
Concentrate	7.04	7.04	7.04	7.04	-	-
Oil-blend	0.00	0.36	0.72	1.08	-	-
Total DM	19.04	18.23	18.43	19.69	0.68	0.31

Note: [1.] Values are expressed as LS Means and standard error of least squares means (SEM). [2.] Treatment effect. [3.] Consociated pasture containing Bromus unioloides and Trifolium pratense.

Feeding the free oil-blend could adversely affect ruminal NDF digestion [41] and reduces DM intake [42] and milk production [43,45]. Effects of free oil feeding on ruminal digestion are variable, including negative [42], neutral [16,46,49] or even positive effects [50,51]. Inclusion of LiOi at 3.2% (± 1.7) or SoOi at 2.9% (± 1.2) of total DM did not affect DM intake [34,36,37]. The forage concentrate ratio (F:C) seems to interact with effects of free oil supplementation on ruminal digestion [52]. When LiOi was included at 3% of DM in a F:C ratio of 65:35, positive effects on NDF digestion were observed with an opposite result when the F:C ratio was 35:65 [51]. In the present trial, the F:C ratio averaged 61:39 (Table 3) and DM intake was not affected.

Estimated intake of linoleic and linolenic acids from the oil-blend was 149.7 and 71.9 g/cow/day in Tr2%, 299.4 and 143.8 g/cow/day in Tr4% and 449.1 and 215.7 g/cow/day in Tr6%, respectively. As negative effects on total DM intake were not observed (Table 3), energy intake would have been higher in oil-supplemented cows but yields of milk or 4%FCM remained unchanged (Table 4).

Table 4. Milk production and composition from grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatment[1]				SEM	*P*-value		
	Control	Tr2%	Tr4%	Tr6%		Treat[2]	Lin[3]	Quad[3]
Milk, kg/cow/day	21.96	20.58	23.99	24.30	1.90	0.46	NS	NS
4%FCM4, kg/cow/day	19.32	17.93	20.61	19.45	1.62	0.73	NS	NS
Fat, kg/cow/day	0.706	0.641	0.724	0.652	0.06	0.73	NS	NS
Fat, g/100g	3.20[a]	3.14[a]	3.06[ab]	2.67[b]	0.14	0.06	0.05	NS
Protein, kg/cow/day	0.831	0.774	0.919	0.963	0.06	0.11	NS	NS
Protein, g/100g	3.81	3.80	3.87	3.99	0.11	0.56	NS	NS
Lactose, g/100g	4.80	4.85	4.93	5.06	0.07	0.08	NS	NS
Total solids, g/100g	12.74	12.85	12.82	12.61	0.23	0.85	NS	NS
Urea, mg/dl	37.83	37.11	33.30	33.12	1.76	0.14	NS	NS

Note: [1]Values expressed as least squares means and standard error of least squares means (SEM). [2]Treatment effect. [3]Contrasts: linear (Lin) and quadratic (Quad). a,b Means in the same row with different superscripts differ significantly ($P < 0.05$). NS = Not significant effect.

Supplementation at 4% of total DM intake with SoOi or LiOi alone or in combination (50-50) increased milk production (+16.7%) without differences between both oils [36]. In our previous trial, the average increase in milk production after oil feeding over unsupplemented cows was moderate (9.4%) and mainly explained by SoOi-LiOi mixtures at a ratio of 75-25 [6]. In non-grazing trials, a high frequency of favorable effects on milk production after the inclusion of unprotected vegetable oils in the diet was reported [53]. Feeding LiOi at 3 or 4% of DM increased milk yield [30] a result that was no observed in other experiments [34,54]. When SoOi was fed at 2.9 (± 1.3)% of the DM ration (0.533 ± 0.228 kg/cow/day) milk yield was not affected in the experiments reviewed by [37] and also when SoOi was supplied at 3.5 to 5% of total DM intake [55,57]. Supplementation with SoOi (1 to 7% of total DM) did not affect milk production [34,35,37,55].

Milk fat content decreased linearly ($P < 0.05$) as intake of the oil-blend increased (Table 4) an effect mainly explained by the significant decrease (-13%) observed in Tr6%. The significant reduction in the concentration of *de novo* synthesized MF-A (-100g/kg) in Tr6% compared to Control (Table 7) was not apparently compensated by a concomitant increase in mammary uptake of the preformed MF-A (+137 g/kg) since milk fat content was lower (Table 4). The result was in turn consistent with the highest concentration of the *trans*-10 $C_{18:1}$ isomer in milk (Table 7) since both parameters correlated negatively (Figure 1).

A direct relationship between increasing levels of *trans*-10 $C_{18:1}$ in milk and the reduction of *de novo* MF-A mammary synthesis has been reported [58] which contributes to explain the linear drop in milk fat content (Table 4). The observed fall in milk fat content was explained in part by the lower presence of the hypercholesterolemic MF-A (Table 7), which improves the healthy value of milk.

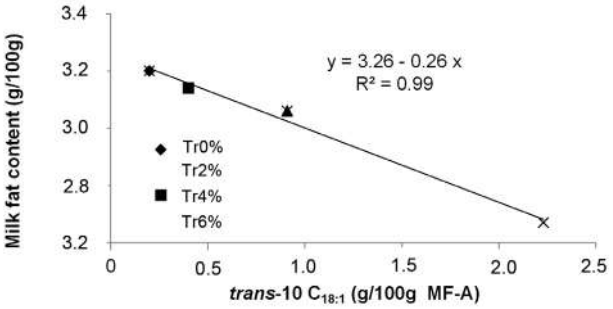

Figure 1. Relationship between milk fat content and *trans*-10 $C_{18:1}$ in milk from cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake

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The decrease in milk fat content after PUFA oil intake is a well-documented result [36,55,57,59,60]. In an extended range (0.2 to 1.0 kg/cow/day) of unsaturated lipid supplementation to grazing dairy cows, an average decrease of of 8% in milk fat concentration and secretion has been reported [17]. Mammary uptake of certain preformed FA (*trans*-10 $C_{18:1}$, *trans*-10, *cis*-12 CLA and *trans*-8, *cis*-10 CLA) reduces the activity and/or expression of genes encoding important enzymes involved in the capture, synthesis and desaturation of MF-A [61] contributing to explain the reduction in milk fat content.

Yield of 4%FCM was not different between treatments (Table 4) suggesting that the numerical increase in milk production in Tr4% and Tr6% compensated for the reduction in milk fat content. These results were consistent with the effects of unsaturated lipid supplementation that generally shows neutral effects on 4%FCM yield in confined [62] and in pasture-based diets [17].

The lack of negative effects on milk protein concentration (Table 4) was an important result since this parameter positively affects the price of milk and determines the speed and quality of milk coagulation for cheese making. In pasture based diets, lipid supplementation does not usually affect milk protein concentration [17,63] while in confined feeding systems this parameter is systematically affected [62,64]. Inclusion of LiOi in the ration of dairy cows does not seem to affect milk protein content or yield [34,36,44,54]. In confined conditions, supplementation with unprotected lipids negatively affected milk protein content in 71% of the cases analyzed by [53] and the result was also associated with a reduction in casein synthesis [65,66]. A large number of studies demonstrated a negative effect of supplemental lipids on the protein concentration of bovine milk [56,60,62,67]. The effect seems more consistent with the use of saturated fats (-0.18 g of protein/100g of milk) and calcium salts of FA (-0.12 g of protein/100g of milk) with respect to unsaturated vegetable oils [62]. From the analysis of 8 supplementation trials feeding unsaturated lipids to grazing dairy cows, a decrease (-3.2%) in milk protein concentration (-0.11 g/100g) was reported [17]. The physiological mechanisms that explain this reduction are not fully elucidated. Some alteration in ruminal fermentation that reduces microbial protein synthesis and therefore amino-acid availability for the mammary gland for milk protein synthesis has been proposed [70]. A dilution effect after milk production increase has also been suggested [17,62,64,70]. In the present work, the increased levels of the oil-blend intake did not affect milk production, milk protein content or yield (Table 4).

Lactose content was also not affected ($P > 0.05$) after intake of the oil-mixture (Table 4). The apparent decrease in *de novo* mammary lipogenesis (Table 7) implies some reduction in glucose oxidation for NADPH synthesis which could have been spared glucose and increased its bioavailability for lactose synthesis an effect that does not appear to have occurred in the present work. Indeed, plasma circulating glucose levels did not change after oil feeding (Table 5). The lack of changes in milk lactose content would be explained by its osmoregulatory capacity. Some authors suggest that changes in lactose content would only occur in very extreme and infrequent feeding situations [71]. Milk urea concentration was not affected by increasing oil intake, a result consistent with the absence of a depressing effect of supplemental lipids on pasture intake (Table 3).

3.3 Concentration of Plasma Metabolites and Changes in Body Weight

Plasma circulating levels of urea, triglycerides, cholesterol, glucose and NEFA were not affected (Table 5).

Table 5. Plasma metabolite concentration in grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatment[1]				SEM	*P*-value[2]
	Control	Tr2%	Tr4%	Tr6%		
Urea, mg/dl	45.87	39.50	44.24	48.05	3.88	0.45
Triacylglycerides, Mmol/L	0.25	0.30	0.30	0.31	0.02	0.41
Cholesterol, mg/dl	220.97	235.44	257.1	252.31	14.54	0.29
Glucose, mg/dl	71.92	70.60	69.13	69.38	3.12	0.91
NEFA, µeq/L	276.71	274.93	280.89	335.38	30.94	0.48

Note: [1]Values expressed as least squares means and standard error of least squares means (SEM). [2]Treatment effect. NEFA= non-esterified fatty acids.

The absence of negative effects on glycemia suggests that the availability of gluconeogenic precursors was not affected by lipid intake which is compatible with the absence of an isoenergetic replacement of carbohydrates by oil in the concentrate and with the lack of negative effects of the oil-blend on concentrate or total DM intakes (Table 3). When total intake was not affected plasma glucose levels remained constant after protected lipid intake, [72]. Even when DM intake was decreased by duodenal infusion of rapeseed oil, glycemia remained unchanged in early or mid-lactation dairy cows [73]. The linear reduction observed in concentration of *de novo* synthesized FA (Table 7) suggests a lower mammary lipogenesis which may have contributed to maintain plasma glucose levels due to a lower oxidation of glucose for NADPH production at the mammary level [73].

Increases in the circulating levels of all plasma lipids after lipid supplementation is a well-documented result

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[74,75] explained by the increase in all fractions of plasma lipoproteins [65,74]. The only exception are triacylglycerides due to their high turnover rate [74] which could explain the lack of effect of the increasing supply of the oil mixture on triglyceridemia (Table 5).

Plasma NEFA was not affected by supplemental lipids a result that was consistent with the positive LW changes observed in all treatments (Table 6) and also with other experiments [77,80].

Table 6. Bodyweight (BW) changes in grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatments[1]				SEM	*P*-value			
	Control	Tr2%	Tr4%	Tr6%		Trat[2]	Lin[3]	Quad[3]	Cub[3]
Initial BW, kg	599.00a	521.50b	556.40ab	538.71b	18.29	0.04	0.50	0.12	0.07
Final BW, kg	647.83a	554.83b	598.80ab	584.00bc	18.78	0.02	0.60	0.05	0.04
Daily BW gain, kg	1.62a	1.12b	1.42b	1.51ab	0.11	0.02	0.40	0.03	0.07
ΔBW, kg	48.83a	33.33b	42.30ab	45.29a	3.41	0.03	0.43	0.01	0.07

Note: [1]Values expressed as least squares means and standard error of least squares means (SEM). [2]Treatment effect. [3]Contrasts: linear, quadratic and cubic. [a,b] Means in the same row with different superscripts differ significantly for treatments effect with P-value as mentioned in column for significance at p<0.05 (Test Tukey-Kramer). ΔBW=final BW – initial BW.

3.4 Milk Fatty Acid Profile

Compared to Control, milk concentration of butyric acid ($C_{4:0}$) resulted lower ($P < 0.05$) only after the maximum oil-blend dose at Tr6% (Table 7). Concentration of $C_{4:0}$ is generally not affected by lipid intake since it is synthesized by an independent malonyl-CoA pathway and therefore not associated with the acetyl-CoA carboxylase activity that is inhibited by the uptake of exogenous FA from oils [1,81]. Any reduction in the levels of $C_{4:0}$ in milk is undesirable for its beneficial effects on human health [3]. In this context, intake of the oil-blend at 4% of the total DM would be the maximum recommended dose.

With respect to Control, the decrease in the concentration of *de novo* synthesized MF-A ($C_{4:0}$ to $C_{15:1}$) was significant only from the Tr4% dose (Table 7). The results reported in [6] also showed a reduction (-22.4%) in the total *de novo* synthesized MF-A from 21.07 to 16.35 g/100g when the same oil mixture was fed at 0.8 Kg/cow/day to grazing dairy cows. A reduction in total concentration of *de novo* synthesized FA from 22.49 to 18.48 g/100g of total MF-A (-18.8%) was aldso reported in grazing dairy cows that consumed 0.7 kg/cow/day of a 70:30 blend of SoOi and LiOi [80]. These effects are explained by the inhibition of the activity of the mammary lipogenic enzymes such as acetyl-CoA carboxylase [65,76] and are normally reported when dairy cows are supplemented with sources of PUFA [82,83]. The inhibitory effect becomes more potent as the length of the PUFA chain and the degree of unsaturation increases and with the presence of double bonds of *trans* configuration [43].

Since at the higher dose (Tr6%) of the oil-blend intake milk fat content was affected (Table 4), the inclusion of the Tr4% dose would be suitable in order to maintain the commercial milk value in a context of payment by quantity of useful (fat-protein) milk solids. At higher oil doses, the decrease in mammary *de novo* MF-A synthesis did not appear to be compensated by a proportional increase in preformed exogenous FA uptake and milk fat content decreased (Table 4). Milk fat depression was maximal in Tr6% with the highest *trans*-10 $C_{18:1}$ concentration in milk fat (Table 7). This *trans*-10 isomer has showed deleterious effects on human health [84] and is negatively correlated with milk fat concentration (Figure 1). A high *trans*-10 $C_{18:1}$ content or the isomer *trans*-10, *cis*-12 $C_{18:2}$ CLA in milk has been related to dysfunctions in the activity of the lipoprotein lipase (LPL) and stearyl CoA desaturase (SCD) enzymes involved in fat synthesis thus causing a decrease in milk fat content [82].

Concentration of total hypercholesterolemic MF-A ($C_{12:0}$ to $C_{16:0}$) decreased significantly with the Tr4% oil mixture without an additional reduction at the higher Tr6% dose (Table 7). At the same time, the atherogenicity index (AI) of milk decreased at the Tr4% dose without additional detriments ($P > 0.05$) between the Tr4% (1.18) and the Tr6% (0.95) doses. Feeding 0.8 Kg/cow/day of the SoOi75:LiOi25 mix showed a 41% decrease in the AI compared to the basal value of 1.93 recorded in the Control treatment in [6]. In the present work, the basal AI was 1.87 (Table 7) being thus comparable to that observed in [6].

Feeding 0.8 Kg/cow/day of the 75% (SoOi)-25% (LiOi) mix induced significant reductions in the $C_{12:0}$ (-30.6%), $C_{14:0}$ (-28.8%) and $C_{16:0}$ (-21.9%) in the experiment by [6]. In the present trial, concentration of $C_{12:0}$ and $C_{16:0}$ showed the same response pattern (Table 7). Compared to Control, concentration of myristic ($C_{14:0}$) acid showed a 21.6% reduction at the T4% dose (Table 7) a result of concern due to the putative atherogenic role the $C_{14:0}$ MF-A [2] when consumed in excess. The observed reductions registered in Tr4% dose for milk content of $C_{12:0}$ (35.9%), $C_{14:0}$ (21.7%) and $C_{16:0}$ (15.5%) were slightly lower than the range estimated in the meta-analysis by [37] when supplementing with SoOi and LiOi. They reported values of 42-37% for $C_{12:0}$., 23-24% for $C_{14:0}$ and 30-17% for $C_{16:0}$.

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Table 7. Milk fatty acid (MF-A) composition from grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of Total DM.

MF-A g/100g MF-A	Treatment[1]				SEM	*P*-value			
	Control	Tr2%	Tr4%	Tr6%		Treat[2]	Lin[3]	Quad[3]	Cub[3]
$C_{4:0}$	2.81[a]	2.36[ab]	2.33[ab]	1.84[b]	0.21	0.02	0.02	0.92	0.35
$C_{6:0}$	1.73[a]	1.40[a]	1.21[ab]	0.91[b]	0.13	0.001	0.001	0.91	0.65
$C_{8:0}$	1.03[a]	0.85[a]	0.64[b]	0.45[b]	0.08	<.0001	<.0001	0.96	0.85
$C_{10:0}$	2.49[a]	2.12[a]	1.46[b]	1.08[b]	0.18	<.0001	<.0001	0.97	0.51
$C_{12:0}$	3.04[a]	2.77[a]	1.95[b]	1.58[b]	0.19	<.0001	<.0001	0.81	0.28
$C_{14:0}$	10.90[a]	10.50[a]	8.54[b]	7.09[c]	0.40	<.0001	<.0001	0.20	0.26
$C_{14:1\ cis\text{-}9}$	1.21[a]	1.35[a]	0.92[ab]	0.95[ab]	0.12	0.03	0.01	0.96	0.05
$C_{16:0}$	24.99[a]	24.55[a]	21.11[b]	20.51[b]	0.72	0.0002	<.0001	0.91	0.08
$C_{16:1}$	0.89	1.23	0.95	1.17	0.13	0.22	0.97	0.67	0.07
$C_{17:0}$	0.45[a]	0.59[a]	0.53[ab]	0.29[ac]	0.06	0.01	0.09	0.005	0.94
$C_{18:0}$	11.50	9.67	11.58	11.06	0.69	0.20	0.29	0.34	0.06
$C_{18:1}$ Isomers									
trans-9	0.21[a]	0.40[b]	0.64[c]	0.73[d]	0.05	<.0001	<.0001	0.32	0.43
trans-10	0.20[a]	0.40[b]	0.91[c]	2.23[d]	0.17	<.0001	<.0001	0.003	0.52
trans-11 (VA)	3.63[a]	4.97[b]	7.05[c]	8.38[d]	0.24	<.0001	<.0001	0.98	0.18
Total trans	4.04[a]	5.77[b]	8.60[c]	11.35[d]	0.34	<.0001	<.0001	0.15	0.45
cis-9 $C_{18:1}$	25.74[a]	26.04[a]	27.90[a]	30.06[b]	0.20	0.005	0.002	0.28	0.74
cis-11 $C_{18:1}$	1.57[a]	1.36[a]	1.94[ab]	1.91[ab]	0.170	0.06	0.01	0.60	0.07
$C_{18:2}$ (n-6)	1.98[a]	2.60[b]	2.81[b]	2.78[b]	0.20	0.03	0.02	0.12	0.88
$C_{18:3}$ (n-3)	0.58	0.78	0.66	0.64	0.06	0.40	0.73	0.18	0.28
cis-9, *trans*-11 $C_{18:2}$ CLA (RA)	2.28[a]	3.16[b]	3.88[c]	3.89[c]	0.22	<.0001	<.0001	0.05	0.57
Short chain MF-A[4]	8.06[a]	6.72[a]	5.63[ab]	4.27[bc]	0.55	0.0005	0.0002	0.98	0.83
Medium chain MF-A[5]	43.33[a]	42.67[a]	34.99[b]	32.48[b]	1.09	<.0001	<.0001	0.40	0.02
Long chain MF-A[6]	48.14[a]	50.03[a]	58.40[b]	62.33[c]	1.32	<.0001	<.0001	0.44	0.08
Saturated MF-A (SMF-A)	60.69[a]	52.38[b]	47.59[c]	43.38[d]	1.15	<.0001	<.0001	0.08	0.57
Unsaturated MF-A (UMF-A)	38.52[a]	42.93[b]	48.69[c]	53.39[d]	1.04	<.0001	<.0001	0.90	0.64
SMF-A/UMF-A	1.59[a]	1.23[b]	0.98[c]	0.82[cd]	0.06	<.0001	<.0001	0.12	0.96
AI[7]	1.87[a]	1.64[a]	1.18[b]	0.95[bc]	0.09	<.0001	<.0001	0.99	0.26
Δ9D products	34.96[a]	38.22[b]	44.30[c]	49.02[d]	1.06	<.0001	<.0001	0.49	0.40
Substrates[8]	55.65[a]	54.41[a]	52.51[b]	52.29[bc]	0.65	0.003	0.0005	0.44	0.43
Índex[9]	0.38[a]	0.41[b]	0.46[c]	0.48[d]	0.009	<.0001	<.0001	0.95	0.40
De novo MF-A ($C_{4:0}$ to $C_{15:1}$)	24.76[a]	22.82[a]	17.90[b]	14.70[c]	1.01	<.0001	<.0001	0.53	0.31
Preformed MF-A (>$C_{17:0}$)	48.89[a]	50.61[a]	58.93[b]	62.62[c]	1.33	<.0001	<.0001	0.46	0.07
Ω6/Ω3	3.57[a]	3.37[a]	4.41[b]	4.63[c]	0.32	0.02	0.003	0.52	0.16
RA/VA	0.56[a]	0.59[a]	0.49[b]	0.37[c]	0.03	<.0001	<.0001	0.01	0.32
∑($C_{12:0}$ to $C_{16:0}$)	38.93[a]	37.81[a]	31.59[b]	29.18[b]	1.15	<.0001	<.0001	0.57	0.10

Notes: [1] Values expressed as least squares means and standard error of least squares means (SEM). [2]Treatment effect. [3]Contrasts: lineal y cuadratic and cubic. [a,b] Means in the same row with different superscripts differ significantly for treatments effect with P-value as mentioned in column for significance at P<0.05 (Test Tukey-Kramer). [4]Short chain MF-A ($C_{6:0}$ to $C_{10:0}$).[5]Mediun chain MF-A ($C_{12:0}$ to $C_{17:1}$).[6]Long Chain MF-A ($C_{18:0}$ to $C_{22:6}$). [7]Atherogenicity index ($C_{12:0}$ + 4*$C_{14:0}$ + $C_{16:0}$)/(∑UMF-A). UMF-A= cis-9 $C_{14:1}$, $C_{16:1}$, cis-9 $C_{18:1}$, cis-11 $C_{18:1}$, trans-11 $C_{18:1}$, $C_{18:3}$, $C_{18:2}$, cis-9, trans-11$C_{18:2}$ (CLA). The detrimental MF-A *trans*-6, 8, 9, 10 $C_{18:1}$ was excluded. [8]Substrates: $C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$ + *trans*-11$C1_{8:1}$. [9]Index: ([∑ Δ9D poducts]/ [∑ Δ9D products + Substrates]).

The level of stearic acid ($C_{18:0}$) did not differ ($P > 0.05$) from Control in any of the oil-blend doses used with a significant and linear ($P < 0.01$) increase of linoleic acid ($C_{18:2}$). These results could be explained by a possible inhibition in the biohydrogenation from $C_{18:2}$ to $C_{18\ 0}$ when high levels of linoleic acid are present in rumen [86,88]. The absence of increases in milk content of $C_{18:0}$ after supplementation with oils rich in $C_{18:2}$ or $C_{18:3}$ can be considered a positive result due to its potential thrombogenic role [2] and was consistent with other experiments [69,80].

The level of oleic acid (*cis*-9 $C_{18:1}$) increased ($P < 0.05$) over Control only in Tr6% at maximum dose of the oil mixture (Table 7). Using the same SoOi-LiOi mixture at a dose of 0.8 kg/cow/day, the oleic acid content (g/100g MF-A) in Control milk (26.14) did not differ from that observed in supplemented cows (27.50) [6]. This result was consistent with that obtained in the Tr4% treatment (Table 7). Differences in oleic acid levels were also not detected

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after feeding 0.7 Kg/cow/day of a 70% (SoOi)-30% (LiOi) mixture to grazing dairy cows [80]. However, the increase in milk oleic content after supplementation with sunflower or SoOi oils is a frequently reported result [37,44,89] even when LiOi is fed [35-37,90].

The linear ($P < 0.0001$) increase in the desaturation index used to estimate mammary desaturation activity (Table 7) was compatible with the increase in oleic acid, contributing in part to maintaining similar levels of $C_{18:0}$ in milk (Table 7). A higher desaturase index was reported in the milk of animals supplemented with LiOi despite the fact that PUFA feeding inhibits the activity of the Δ9-D enzyme complex [45]. Several authors did not observe differences in this index when comparing a control ration with those that included SoOi and LiOi or their mixtures at 50-50 [36].

Milk fat content of linoleic acid increased ($P < 0.02$) linearly from 1.98 (Control) to 2.78 g/100g MF-A in Tr6% (Table 7) thus remaining within the normal range (2-3 g/100g MF-A) reported by [1] but lower than that observed in our previous experiment (3.25 to 3.92 g/100g MF-A) after supplementation with 0.7 Kg/cow/day of a mixture of 70% (SoOi)-30% (LiOi) in grazing cows [80]. Likewise, an increase in milk $C_{18:2}$ content from 1.96 (Control) to 3.50 g/100g MF-A was reported after intake of the same SoOi LiOi mixture at 0.8 Kg/cow/day [6].

On the other hand, the levels of linolenic acid ($C_{18:3}$) in milk did not differ ($P > 0.05$) between treatments (Table 7), a result consistent with that observed in [80]. Feeding LiOi at 4% of DM intake in pure form increased (170%) the levels of $C_{18:3}$ in milk a result not observed when cows were supplemented with a 50% mixture with SoOi [36].

The milk Ω6/Ω3 ratio from Control cows was low (3.57) and increased ($P < 0.02$) with the increasing supply of the oil mixture but always remaining below the recommended value of 5. When the same oil mixture was fed at 4% of total DM intake to grazing dairy cows, the Ω6/Ω3 ratio remained between 3.18 in control milk and 3.87 in supplemented cows [80]. In order to achieve a low Ω6/Ω3 ratio, the supply of LiOi alone at 4% of total DM intake was very effective averaging 2.13 in supplemented vs. 4.25 in control cows. The effect was attenuated when using mixtures with SoOi [6]. In that work [6], the milk Ω6/Ω3 ratio after intake of 0.8 Kg/cow/day of the 75% (SoOi)-25% (LiOi) blend was greater (5.66) tan observed in the present experiment.

Feeding non-protected PUFA oils increase synthesis of differents isomers of *trans*-$C_{18:1}$ MF-A in the rumen which are transferred to milk. Some of them, like the *trans*-9 (elaidic) and *trans*-10 $C_{18:1}$, are classed as deleterious or unhealthy [85,91,92] and hence any excessive increase in concentration in milk should be avoided. Basal concentration (g/100g MF-A) of *trans*-9 (0.21) and *trans*-10 $C_{18:1|}$ (0.20) was linearly ($P < 0.01$) increased after oil-blend intake (Table 7) reaching máximum values of 0.73 for *trans*-9 and 2.23 for *trans*-10 $C_{18:1}$ in Tr6% that can be considered low or harmless. Indeed, a concentration of 2.28 g/100g MF-A of *trans*-10 $C_{18:1}$ in a butter supplied at 12% of the diet of experimental rabbits subjected to a cholesterol challenge did not show deleterious effects on the plasma lipid profile or the metabolism of lipoproteins when the level of *trans*-10 $C_{18:1}$ was accompanied by at least 7 g/100g MF-A of VA and 3 of RA [85]. In Tr4%, the *trans*-9 (0.64 g/100g of total MF-A) and *trans*-10 $C_{18:1}$ (0.91 g/100g MF-A) concentrations in milk fat were lower ($P < 0.05$) than in Tr6% showing some advantage. Since in Tr4%, concentrations of *trans*-10 $C_{18:1}$ were only 0.91 g/100g MF-A of the total MF-A with levels of VA and RA in milk of 7.05 and 3.88 g respectively (Table 7) it can be expected an athero-protective role of Tr4% milk similar (or even higher) to that obtained in [85]. Intake of 0.7 kg/cow/day of a 70% (SoOi)-30% (LiOi) blend also induced low values of *trans*-9 (0.58 g/100g of total MF-A) and *trans*-10 $C_{18:1}$ (0.99 g/100g of total MF-A) in milk from grazing dairy cows [80]. In Control milk, concentration of VA accounted for 90% of the total *trans*-$C_{18:1}$ MF-A a value that remained high (74 to 86%) after intake of the oil-blend doses (Table 7). In Control milk, *trans*-9 and *trans*-10 $C_{18:1}$ represented 5.19 and 4.95% of total *trans*-$C_{18:1}$ but the relative contribution of the *trans*-10 $C_{18:1}$ increased after feeding the oil-blend. The relative increase expressed as % of the total *trans*-$C_{18:1}$, resulted greater in treatments Tr4% (10.5%) and Tr6% (19.7%). Although the concentrations of these two *trans* FA were moderate, it is convenient to avoid deviations towards its formation due to its potential atherogenic effect [84,85]. It seems therefore advisable not to use doses greater than 4% of supplementary lipids in order to avoid non-undesirable deviations towards non-healthy trans isomers appearance.

The concentration of VA in milk showed a linear increase ($P < 0.01$) after increasing the oil mixture intake but its apparent conversion into RA estimated by the RA/VA ratio showed an opposite ratio reaching a minimum value of 0.37 in Tr6% (Table 7). The result suggests that the increase in precursor availability (VA) for RA synthesis did not induce proportional increases in the activity Δ-9D desaturase which was also consistent with the lack ($P > 0.05$) of increase in milk RA content between treatments Tr4% (3.88 g/100g MF-A) and Tr6% (3.89 g/100g MF-A).

An average RA/VA ratio of 0.41 was proposed by [93] a value that resulted close to the 0.49 observed in Tr4%

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(Table 7). VA present in milk and dairy products can exert beneficial properties in itself through a direct anticarcinogenic effect [94] or mediated after its endogenous conversion to RA at an estimated rate of 20% in human tissues [95] via Δ9-desaturase activity [93].

Since only 20% of the VA would be converted into RA in human tissues and until more experimental evidence of the healthy effects of VA is available, it seems advisable to avoid excess milk VA concentration and intake. In this context, our results suggest that the oil-blend fed at 4% of total DM intake (Tr4%) would be the most advisable dose since marginal increases in RA were not detected when VA increased with higher oil-blend intake up to Tr6% (Table 7 and Figure 2).

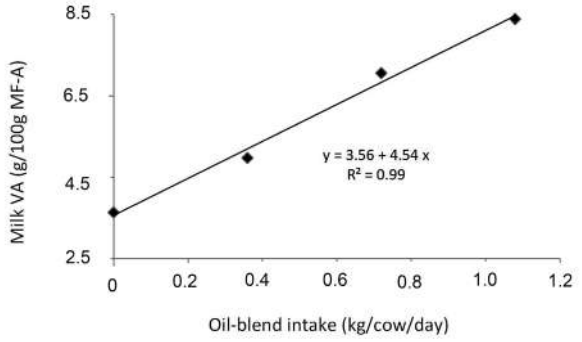

Figure 2. Concentration of vaccenic acid (*trans*-11 $C_{18:1}$) in milk from grazing dairy cows supplemented with increased levels of a soybean-linseed oil blend (75:25)

As expected, milk RA concentration correlated positively (R^2 = 0.89, P < 0.01) with VA (Figure 3), a frequently reported result [1,36]. The average conversion rate of VA into RA appeared to be 34.2% (Figure 3) that resulted very close to the 35-39% values estimated at [80] after supplying 700 g/cow/day of a mixture of 70% (SoOi)-30% (LiOi) blend to grazing dairy cows. Taking the RA/VA ratio as an estimator, the average conversión rate resulted somehaw higher (50.25%, Table 7).

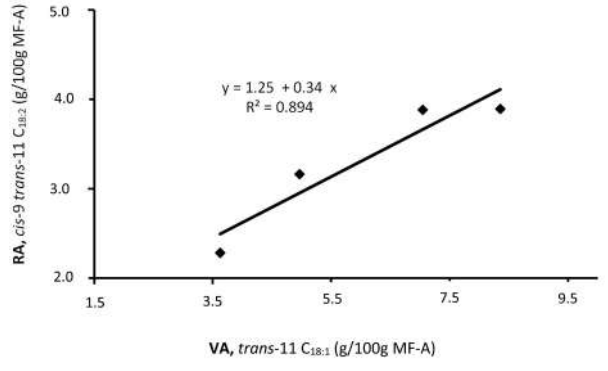

Figure 3. Concentration of vaccenic acid (*trans*-11 $C_{18:1}$) in milk from grazing dairy cows supplemented with increased levels of a soybean-linseed oil blend (75:25)

In the present work, the baseline (Control) concentration of RA resulted very high (2.28 g/100g MF-A) and was increased (P < 0.05) after oil-blend intake without differences (P > 0.05) in RA concentration between Tr4% and Tr6% (Table 7). This result suggests that the response in milk RA content would be linear up to a maximum of 4% of oil-blend consumption (Figure 4) and confirms what was previously suggested by [1].

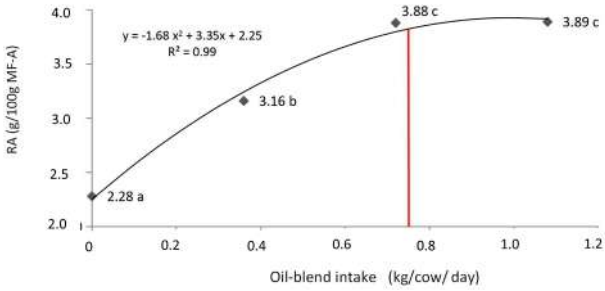

Figure 4. Concentration of rumenic acid (RA, *cis*-9 *trans*-11 $C_{18:2}$) in milk from grazing dairy cows supplemented with increased levels of a blend of soybean and linseed oil (75:25)

At a similar oil-dose (4% DM intake), milk concentration of RA observed in Tr4% (3.88 g/100g MF-A) resulted higher than those reported in [36] (1.60-2.39 g/100g MF-A) in rations with a high forage content (59%) and also than those obtained in [6] using the 75% (SoOi)-25% (LiOi) oil blend (3.21 g/100g MF-A). When grazing dairy cows were fed 0.7 Kg/cow/day of a mixture of 70% (SoOi)-30% (LiOi), milk concentration of RA averaged 3.13 g/100g MF-A [80].

Milk content of RA in Tr4% (Table 7) was also higher than values reported in the meta analysis by [37] after feeding SoOi (1.02 (± 0.36) g/100g MF-A) or LiOi (1.75 (± 0.84) g/100g MF-A) and that those reported in [44] when the cows were supplemented with 0.5 kg/day of sunflower or soybean oil (2.02 g/100g MF-A) or after feeding 0.9 kg/cow/day of unsaturated FA calcium salts [98]. The high baseline values of RA observed in Control milk (2.28 g/100g MF-A, Table 7 and Figure 3) could partly explain the differences between experiments.

The presence of unsaturated MF-A increased linearly (P < 0.0001) with oil intake reaching a maximum at Tr6% with a 38.6% increase over Control (Table 7). The SMF-A/UMF-A ratio decreased (P < 0.0001) with oil intake without differences between Tr4% and Tr6% (Table 7). The results obtained confirmed the existence of great response plasticity in the composition of milk fat in terms of its constitutive MF-A [1,37] which can be modulated by oil supplementation to increase the healthy value of dairy products.

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4. Conclusions

Supplementation of grazing dairy cows with increasing doses of a mixture of soy and linseed oils linearly increased the rumenic and vaccenic acid content of milk without significant deviations towards unhealthy fatty acids like *trans*-9 or *trans*-10 $C_{18:1}$ and without affecting either milk production or protein content. This nutritional strategy was also an effective tool to reduce milk content of saturated milk fatty acids and the hypercholesterolemic fraction of milk fat which improves its healthy value. Overall results shows that the optimum level of inclusion of the soybean-linseed oil mixture was around 3.91% of total DM intake of cows without additional advantages by increasing the dose of oils in the total ration.

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ARTICLE

Five years Retrospective Study of Avian Coccidiosis in a Veterinary Clinic Bukuru Plateau State Nigeria

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ABSTRACT

This study was conducted to evaluate the incidence of avian coccidiosis and its associated various risk factors such as age, type of birds and season in a private veterinary clinic in Bukuru, Plateau State Ngeria. A total of 9406 cases during 2013 – 2017 were analysed and 1556 of them were positive for coccidiosis. There are several reports on the prevalence of avian coccidiosis by previous researchers; however, in this study we evaluated the prevalence of avian coccidiosis in the study area and its economic impacts. Total prevalence of 12.14% in 2013, 18.78% in 2014, 18.21% in 2015, 16.82% in 2016 and 19.07% in 2017 were reported. An overall prevalence of 85.02% was recorded. The average prevalence of coccidiosis based on this five years study is 17%. The association between coccidiosis and age of the birds was determined and age 5-8 weeks becomes most effective period with wet season having high percentage prevalence of coccidiosis. Based on the type of birds, coccidiosis is prevalence almost in equal proportion in both broilers and layers. The losses caused by avian coccidiosis could be both direct and indirect components which may include the cost of control measures, inadequate good hygiene practices, production losses and lack of prophylaxis treatment. The control of avian coccidiosis can be achieved through good sanitary measures by avoiding water spillage on the pen floor, overcrowded stocking density, the use of prophylaxis- anticoccidials and proper good vaccination practices.

1. Introduction

The business of poultry farming in Nigeria has in recent times witnessed enormous expansion [1]. The estimated commercial poultry birds population in Nigeria rose from 110 million at the beginning of this century, to over 150 million in 2006 [6]. In spite of this enormous growth, the poultry industry has suffered a lot of constraints of which poultry disease is a major player in this setback/contraint [5]. Avian coccidiosis in poultry is caused by several species of the genus Eimeria [16]. The disease causes reduced growth, emaciation, anaemia

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and mortality in the infected birds [7]. This could results in heavy economic losses due to the cost of treatment and prevention measures [17]. Warm and humid weather provides favourable conditions for the growth and development of infective ooysts [5] which could result in a high prevalence of coccidiosis in any tropical location like Bukuru.

Avian coccidiosis is caused by protozoan parasite of genus eimeria species of the family *Eimeriadae* and order *Eucoccidiorida* [3]. The parasites develop within the intestine of most infected poultry birds. Seven species of eimeria (*E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox and E. tenella*) are recognised that infect chickens [13,18]. Despite the fact that coccidiosis is a disease known for plenty years, it is still considered as the most economical important parasitic disease condition affecting poultry production world-wide uptil now [15,18]. Coccidiosis has played a major role in economic loses to poultry famers the world wide, for example in 1995 the United Kingdom was estimated to have lost thirty eight million pounds due to avian coccidiosis [10]. Many poultry famers have not taken sanitary measures serious in their poultry business so disease like coccidiosis have had its way in destroying such farms badly [10,15]. Diagnosis of coccidiosis could be based on the history of bloody diarrhea/feces in infected birds or carcasses/moribund birds, with post mortem and microscopy examination considered as one of the best option to be used to confirm its diagnosis. Post mortem examination of infected poultry carcasses may revealed lesions on the serosal and mucosal surfaces of the intestine most times. Some of the lesions observed may include; enteritis of the interior one third, the middle and the posterior one third of the intestine depending on the type of coccidian and these could manifest as hyperemia, necrosis of the intestinal mucosa and bloody feces in the lumen, thus serving as pointer to the presence to the disease, and this could be complimented by microscopy to observe for, gamonts, schizonts and oocysts usually with a lot of successes recorded [11,17]. Although prevalence of coccidiois has been reported in various locations in Nigeria by some researchers [2,9], however, in Bukuru Plateau State there is a dearth in data of Eimeria species and the prevalence of coccidiosis. Hence this study seeks to investigate the prevalence of coccidiosis and Eimeria species in Bukuru Plateau state. It is therefore important to survey for the disease that occur most frequently among poultry farmers in Bukuru and environs through this retrospective study. The aim is to conduct a five years retrospective study of avian coccidiosis in a private veterinary clinic Bukuru, Jos south Plateau state during 2013-2017.

2. Materials and Methods

2.1 Study Area

The study was conducted at ECWA veterinary clinic in Bukuru- Nigeria. It is a private clinic which operates in Bukuru near Jos where diagnosis, treatment and other veterinary service are offered for all classes of livestock and pets. The clinic also undertakes vaccination; meanwhile they provide upon request the feasibility studies for different classes of livestock investment. The clinic has a workforce comprising of two veterinary doctors and many attendants, sales men as well as a security man. The clinic under review has a laboratory, post-mortem room, treatment room, surgery room, three quarantine pens, drug and feed stores and three offices.

Bukuru geographical co-ordinates are 90 48' 0' NORTH, 80 52' 0' EAST.

2.2 Methodology

The record of diseases as presented and diagnosed at the ECWA veterinary clinic Bukuru during the five years (2013-2017) period was retrieved and analysed retrospectively. The diagnosis at the clinic was based on clinical signs and post-mortem findings using the method described by Olabode et al. [13].

2.3 Statistical Analysis

Descriptive statistic frequency and percentage was used for categorical variables. Association between infections and other factors such as types of birds, age, etc. were accessed using chi-square test for the association and odd ratio. $P<0.05$ was considered significant. Descriptive statistic was used to analyse data, tables were used for results and proportions presented in percentages.

3. Results

Table 1. The prevalence of coccidiosis in chickens based on ages

Month of occurrence	1-4 weeks	5-8 weeks	3-6 weeks	> 6months
January	46	32	32	26
February	55	128	121	55
March	43	37	27	27
April	40	46	21	15
May	37	43	22	16
June	57	48	31	19
July	29	36	33	12
August	41	27	22	27
September	40	32	31	25
October	40	49	25	19
November	35	38	27	21
December	35	26	24	25

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Note: Ages 5-8 weeks and 1-4 weeks appeared to be more infective age. Although at age 3-8 months, the prevalence was also recorded higher but only in Februaries in the 5 years study. While ages above 6-months had least infective periods [10].

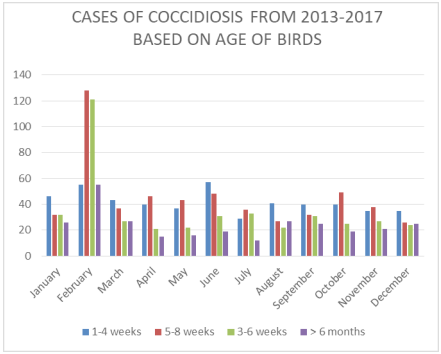

Figure 1. Reports of coccidiosis during 2013-2017 based on the age of birds

Table 2. The association between disease and season

Season			Occurrence of coccidiosis		Total
			present	absent	
wet or dry season	dry	Count	795	3660	4455
		Expected Count	725.1	3729.9	4455.0
		Adjusted Residual	3.9	-3.9	
	wet	Count	731	4190	4921
		Expected Count	800.9	4120.1	4921.0
		Adjusted Residual	-3.9	3.9	
Total		Count	1526	7850	9376
Expected Count		1526.0	7850.0	9376.0	

Note: P<0.05 [P=0.01] df=1 T=15.345 since the P is less than 0.05, it means there is an evidence of significant of association between disease and season in chicken occurring during 2013 to 2017

wet or dry season * coccidiosis or no coccidiosis Cross tabulation

Table 3. Prevalence of coccidiosis during 2013-2017 based on types of birds, (Layers and broilers). Here the infection of coccidiosis is slightly high in the layers than in the broilers with the period under review

BASED ON TYPES OF	BIRDS FROM 2013-2017	
MONTHS	LAYERS	BROILERS
January	64	69
February	177	182
March	70	64
April	64	69
May	66	53
June	75	80
July	58	50
August	60	51
Septembe	66	63
October	60	73
Novembe	61	44
Decembe	36	47
	857	845

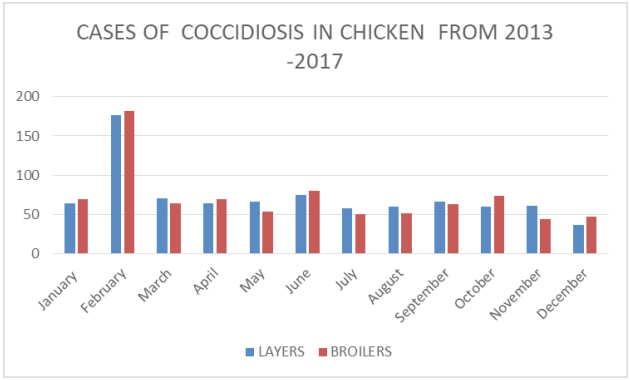

Figure 2. Prevalence of coccidiosis during 2013-2017 based on types of birds

Table 4. The total prevalence of chicken coccidiosis yearly

YEAR	TOTAL PREVALENCE	
2013	12.14	
2014	18.78	
2015	18.21	
2016	16.82	
2017	19.07	

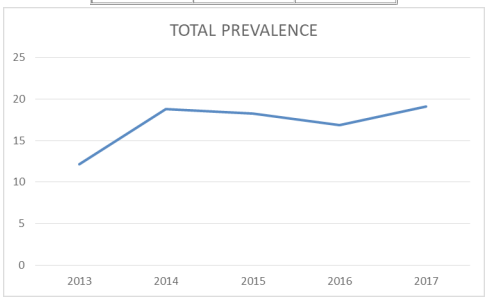

Figure 3. The total prevalence of coccidiosis yearly in chickens

4. Discussion

Based on results of this study, it was observed that age of the birds plays a vital role in infectivity of coccidiosis which is much prevalent in age 5-8 weeks birds as indicated in table 1 and figure 1. The prevalence showed a decline in birds with age 1-4 weeks, though prevalent in age 3-6months birds was highest but only in the months of February throughout the five years study. This means that age 3-6 months and above were the least infective period which could be due to previous exposure of the old birds. This agrees with the report of Chauham and Sushovan [4] and Mark et al. [10]. There is an evidence of significant association between the presence of coccidiosis in chicken and the age group occurring within 2013-2017 where the observed value in the adjusted residual value of coccidiosis presence in chicken with age group 1-8 weeks was

less than what would have been expected through chance alone. While in the other hand the adjusted residual value the observed value of coccidiosis in chicken with age 1-8 group was more than what would expect. Meanwhile, in the adjusted residual value of coccidiosis presence in chicken with age group of > 24 weeks was found to be less than what would expect. The overall results show that the disease is much prevalent in the younger birds. Chickens within ages 1-8 weeks were most diagnosed with coccidiosis. The highest infective period was between 9-24 weeks of age with great declined at age > 24 weeks old, this is in agreement with Chauhan and Sushovan [4] and Mark et al. [10].

Table 2 indicates the association between disease and season ($P<0.05$). There is a significant association between disease and season in chicken occurring during 2013 to 2017. From the adjusted residual value we had more coccidiosis than expected through chance alone in the dry season' also we had in the wet season less observation than we expected through chance alone. *Eimeria* oocysts remain viable in litter for many months, hence they contaminate the farm year in year out thereby maintaining infection of the poultry flock. The *Eimeria* oocysts can killed by freezing and extreme dryness and high temperature, thereby making it difficult for the infection to get spread in the dry and cold month as agreed by [4,5]. Also wet area constitutes a source of avian coccidiosis infection. The poultry pen litters are supposed to be applied in thick layers in order to facilitate maximum absorption of the bird's waste droppings. The wet litters enhances sticking of materials to boots, utensils, shoes, vehicle wheels and clothing leading to a faster rate of transfer of the organism to other farms. Bukuru is a wet and damp place hence the prevalence of coccidiosis among poultry farmers as agreed with the point raised by Chauham and Sushovan [3,4,12].

Table 3 shows prevalence of coccidiosis during 2013-2017 based on types of birds, (Layers and broilers). Here the infection of coccidiosis is slightly high in the layers than in the broilers within the period under review but figure 2 shows that there is no significance difference in coccidiosis prevalence based on the types of birds. This means that coccidiosis is prevalence almost in equal proportion in both boilers and layers in this study. The observed value presence in broilers was more than the expected and this occur by chance alone while compared to the layers where in the adjusted residual value, the value observed of coccidiosis presence in layers occur was more than the expected and this occur by chance alone. There was no significant difference ($p>0.05$) between the prevalence rate of coccidiosis in layers and broilers. This is in agreement with the report of Olanrenwaju and Agbor [14]. In layer and broiler, the prevalence rate of coccidiosis in birds reared under deep litter system and battery cage system of management shows a strong association between system of management and occurrence of the disease with the former system being higher. The higher prevalence rate in broilers and layers reared under deep litter system of management compared with the battery cage system of management is in agreement with the report of Etuk et al. [5], Hadipour et al. [7] and Jatau et al. [8]

Table 4 and figure 3 show the total prevalence of coccidiosis was lower in 2013, having the least of 12.14% prevalence, while in 2014 the prevalence elevated to about 18.78%, and then declined a little bit to 18.21% in 2016. Reasons could be because of adequate awareness in hygiene practice in poultry production. This agrees with the point earlier raised by Chauham and Sushovan [4]. Subsequently the infection became high on 2017 with increase rate of 19.07% which might be due to decrease in the awareness of good bio-security practice.

5. Conclusion

The average prevalence of coccidiosis in this study was 17 %. Age plays a great role in infectivity of coccidiosis with age 5-8 weeks birds showing much prevalent, with wet season having high prevalence. Coccidiosis had prevalence almost in equal proportion in both boilers and layers in present study. Poultry farmers should adhere to good vaccination and hygienic practices as well as the use of both drugs and vaccines to prevent the incidence of the disease. Farmers should adhere to routine chemoprophylaxis and avoid factors of predisposition to cooccidiosis especially during the raining season. A further study of the genetic basis of parasite survival on the hosts and the key molecules associated with the disease would be essential so as combat the disease effectively.

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Competing Interest

The authors declare that they have no competing interests.

Authors' contributions

BIJ and LHB designed the research; ISI, ISE,SA and OVB performed the research and analysed the data; OMO wrote the manuscript; IBS have taken part in the revision of the manuscript. All authors read and approved the final

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ARTICLE

Contribution of Livestock Production to Global Greenhouse Gas Emission and Mitigation Strategies

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ABSTRACT

Understanding the interaction of livestock production and climate change is currently the main issue in global warming. This paper reviews the contribution of livestock production in greenhouse gas emission and its mitigation strategies. The potential contribution of individual large ruminants are 200-500 litters of methane per day while small ruminants produces 20-40 litters of methane per day. The major greenhouse gas related to livestock production are methane and nitrous oxide which contribute approximately about 14.5% global GHG emissions. Limiting emissions from livestock, without compromising food security, is an important limit greenhouse gas emissions. The main choices for reducing greenhouse gas emission in livestock production are more related to improving animal production. Mitigating emission of CH4 by means of improved management of biogas and manure, reducing CH4 emission from enteric fermentation through improved efficiency and diet, husbandry as well as genetic management are some of strategies used in mitigating enteric emission of methane from livestock. The other one is mitigating emission of nitrous oxide through more efficient use of nitrous fertilizer, proper manure management and by using different feed additives.

1. Introduction

Agricultural production potential has previously grown-up 2.1-2.3 in over the last 40 years as reported by [1] and this is responsible for 10-12% of the global anthropogenic greenhouse gas emission [2].

Fossil fuels are the major causes of climate change as first list. Some or the main source of resulted by human related activities as emission of carbon dioxide and some of greenhouse gas are: natural gas, Oil and especially coal. However, the life of animal and animal production as food for human are comprehended that as a main source of greenhouse gases, and this in fact not less than half of human caused greenhouse gases emission [3].

According to the report of [4] there is an expectation of Human population increment from 7.2 - 9.6 billion by the year 2050. This indicates that 33% population increase, but obviously as global living standard increase, the increment in demand for agricultural product will increase by around 70% in the future in the same period estimated by [1]. Livestock is among one of the fastest growing from agricultural subsectors in developing countries. In these country GDP is around 33% share of the total agricultural GDP and is rapidly increasing. This easily shows that progress is induced by the quickly increasing demand for animal products driven by population growth as well as in-

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creasing income and urbanization in developing countries [5]. Ruminants are expected to be an important component of global production and there is a growing demand especially for animal protein sources [6], still keeping their indispensable role in the management and preservation of ecosystems, namely in natural and semi-natural grasslands and rangelands and agrosilvopastoral systems, among others. But, a substantial upsurge in agricultural production will be required to meet these increasing demand for animal originated protein foods [2]. This is an event that is likely to lead to strengthened production practice and following increases in Greenhouse gas emission.

Livestock system have both negative and positive effect on social equity and economic growth, natural resource and public health [7]. Livestock produces greenhouse gases in different forms like: in the form of nitrous oxide (N_2O) from the use of nitrogen containing fertilizer, methane (CH_4) from enteric fermentation and N_2O and CH_4 from livestock manure deposition on the pastureland and from different animal manure management. Carbon dioxide is also produced on different livestock farms from different energy usage and fuels [8].

Currently there is a huge rising interest in understanding the linkage between agricultural production especially livestock production and climate change and it has been motivating a significant amount of research [9]. Therefore, this paper reviews the livestock sector's contribution to the global climate change and its mitigation strategies.

Objectives

To describe the contribution of livestock sector on climate change

To clarify and summarize mitigation strategies

2. Contribution of Livestock Production Practises to Climate Change

Livestock system plays significant role in climate change [10]. Livestock production directly and indirectly contributes about soil carbon loss in grazing land, deforestation for grazing land and intensive animal feed production, the amount of energy used in cultivating and harvesting feed and processing, transporting dairy products, meat and meat products, live animals and animal feed, gases from animal manure (especially CH_4) and enteric fermentation and nitrous oxide (N_2O) releases from the use of nitrogen containing synthetic fertilizers[11]. Greenhouse gases most often associated with animal production are methane, nitrous oxide and carbon dioxide [12, 13]. Similarly, greenhouse gas emission from agricultural sector that are related to animal production are CH_4 which directly emitted from livestock stomach and manure, while nitrous oxide (N_2O) emitted from fertilizer applied soil and manure as well as grazed lands as reported by[14].

Different authors approximate the contribution of livestock production on global greenhouse emission with different figures. [15] estimate the livetock contribution to global anthropogenic greenhouse gas emission at between 7 - 18% . methane is the most important gas produsedin agriculture [13]. Ruminant livestock approximatly can produce 250-500 litter of CH_4 per day. This level of production results in estiation of the contribution large ruminant to global warming that may occur in the next 50-100 yeqrs to be less than 2% about 65 % of the livestock production emissions. With respect to activities, feed processing and production and enteric fermentation from ruminants are the two major sources of emissions, contributing 45 % and 39 % of total emissions respectively. Manure storage and processing forms 10 % and the rest is attributed to transportation and animal processing. On product-basis, milk from cows and beef are responsible for the most emissions, contributing 20 % and 41 % of the sector's total greenhouse gas (GHG[16]. Majority of the livestock industry emission are in the form of methane (44%), while 29% and 27% are nitrous oxide and carbon dioxide respectively (figure 1) below [2,17,18].

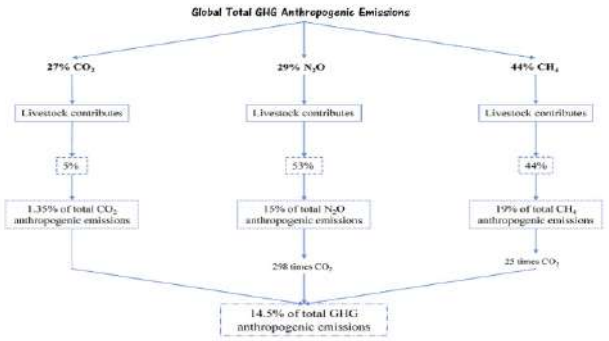

Figure 1. Livestock contribution to global GHG emission [18]

The green plant used up by the livestock instigates from the conversion of atmospheric carbon dioxide to biomass or organic compound. Hence, under the Kyoto Protocol (2005) it is assumed that the amount of consumed carbon dioxide in negative form are equivalent to those emitted by the animals. Therefore, livestock respiration is not counted as a net source of Carbone dioxide emission since they are part of global biological cycle. On the other hand, the animal is thought to be a carbon sink since a fraction of the Carbone consumed is absorbed in the live tissue of the livestock and livestock products like milk and meat [19].

Many authors reported that emission form animal production contribute more greenhouse gas emission to the

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atmosphere than the entire global transportation sector. Thus, the domestic animal donates indirectly and directly to greenhouse gas emission [20, 21].

2.1 Direct Contribution of Livestock to Greenhouse Gas Emission

Some of the direct emission from animal source include animal physiology, respiration, enteric fermentation and excretion [22].

Emission of CH4 is thought as one of the most significant global issue [2]. During feed fermentation and digestion in animals, methane gas is produced as by-product of digestion of structural carbohydrate majorly cellulose due to the action of microorganism (fungus, protozoa and bacteria) in the rumen (figure 2). At the time this digestion of monosaccharide are fermented to CO_2, H_2 and VFA such as propionate, butyrate and acetate [23]. This process releases H_2 while producing VFA and some of the microbial cells comprising energy and essential protein to be made available for the growth of animals in all ruminants, the H_2 is removed through the action a group of microorganism known by methanogenic archaea or methanogens that can gain their energy via combining CO_2 and H_2 to form methane [24]. Of course, CH_4 is produced by archaeal microorganism known as methanogen which utilizes predominantly carbon dioxide and hydrogen in the rumen to form CH_4 in the animals, thereby maintaining the lower partial pressure of H_2 in the rumen. Actually this CH_4 production from the production from the rumen archaea result in 2-12% loss of metabolizable energy in the rumen [16,24]. According to the report of [2] GHG account shows that CH_4 emission from livestock is almost equivalent to the GHG emission from the transportation sector in the case of Australia.

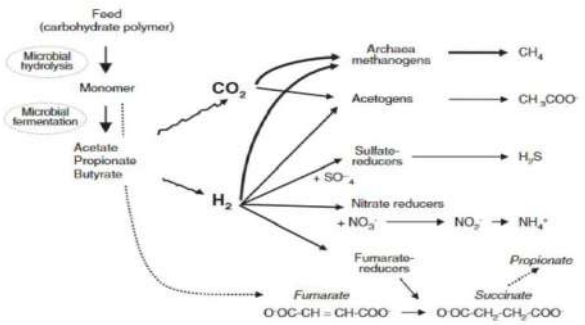

Figure 2. Feed and H_2 reduction in the rumen adopted from [25]

2.2 Indirect Contribution to Greenhouse Gas Emission

Indirect emission refers to emission resulted from manure application, manure storage, farm operation, land application chemical fertilizers and manure treatments, feed crop, transportation, animal product processing and land allocation for livestock production (like: desertification, deforestation and carbon release from cultivated land) [20]. Generally, in the case of livestock production indirect emission play a great role in the release of C_2O to the atmosphere than direct emission [26].

Greenhouse gas emission from animal production in particular and agriculture in general are expected to grow as food production expands to keep pace with a growing world which is expected to reach 8.3 billion by 2030 and 9.1 bill by 2050 as estimated by [4].

In developing country especially in Africa, there is an increase in CH_4 emission resulted from increased livestock production. According to the report of [27] there was an estimation that African cattle, sheep and goat produce about 7.8mil tons of CH_4 in 2000 which are likely to increase to 11.1mil tons by 2030. As [28] reported that, in case this linear relationship between methane emission and livestock population continue, it could be concluded that global methane emission form animals production may increase 60% by the year 2030. Though, the moderate solution for reducing methane emission from livestock production practices could be changing feeding practices and manure management [29].

3. Livestock Sector GHG Mitigation Strategies

Reducing greenhouse gas emissions from livestock, without conceding food security is therefore clearly an important portion of any international effort to limit greenhouse gas emission overall and their effect on climate system [30].

The main alternatives for limiting GHG emission per unit of animal production: firstly, mitigating emission of CH_4 via improved management of biogas and manure; secondly, reducing CH_4 emisssion from enteric fermentation especially in ruminant animals (mostly cattle, goat and sheep) via improved feed efficiency; thirdly, mitigating emission of NO_2 through more effectual use of inorganic or nitrogenous fertilizers; fourthly, confiscating carbon and mitigating CO_2 emission by reduction and reversal of deforestation due to agricultural intensification and by restoration of organic carbon to cultivated soil and degraded pasture land or rangeland and fifthly, changing the herd structure through increasing the proportion of monogstric animals like pig and chickens as well as vegetarian fish in the flow of animals grown for human consumption [31].

3.1 Methane Mitigation Strategies

Numerous studies have formulated reduction schemes to

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mitigate methane emission. Generally, mitigation can be grouped in to two: basically those targeting manure management and those targeted at enteric fermentation [23].

3.1.1 Methane Mitigation Strategies Aimed at Enteric Fermentation

Diminishing enteric CH_4 emission from ruminant livestock without changing livestock production is needed both as a strategy to reduce global greenhouse gas emission and as means of improving feed conversion efficiency of the individual animals [32].

Some of mitigation strategies can be used to reduce greenhouse gas emission such as the use of some specified chemicals and vaccines, genetic selection and the capture of methane have been proposed, yet dietary management is considered the most promising strategy for the diminution of methane from ruminant animal production system [33].

(1) Genetic Management

Naturally the potential of animals to produce enteric methane is vary. As the first strategy to reduce methane emission per individual animals is the use of selection or selective breeding with an animal's permitting low methane emission per unit of feed consumed 10% with no negative impact on productivity record [10]. Therefore, selecting animals that shows excellent production performance on low quality feds is also another way of reducing CH_4 emission per individual animal product.

Another option to reduce methane emission is the potential of changing rumen microorganism. Currently changing the rumen microbial composition in lambs and calves after weaning towards lowering methane emission in the future adult life is being explored and practically available [10].

(2) Dietary Manipulation

Dietary manipulation is also the second strategy to reduce methane emission per individual animals. Harvesting pasture and forage at early maturity stage improves its nutritional content of some soluble carbohydrate and decrease the level of lignin in the plant cell wall thus increases its digestibility [34] and also reducing enteric methane emission per unit of digestible dry Matter.

Mechanical processing of feeds like processing via its influence on energy losses, passage rate and digestibility can be an effective enteric methane emission mitigation alternative although it may not be economically feasible in some animal production systems. Providing higher quality forage is also another way of reducing enteric methane emission because it improve digestibility of the feed [15].

Another strategy of dietary manipulation is concentrate supplementation. Addition of small amount of concentrate to all roughage (natural pasture or forage) is expected to increase animal productivity and reduce greenhouse emission per individual animals [15].

Lipid supplementation is the most reliable and technically acceptable nutritional manipulation used to reduce enteric methane emissions. Nevertheless, its diminution potential is ultimately limited by a restriction on dietary inclusion in order to maintain production efficiency [33]. Similarly, [15] reported that dietary lipid are effective in reducing enteric methane emission, but the application of this practice will depends on its cost and its effect on feed intake, production and product composition like milk composition. Reductions of 10-25% may be achieved via the supplementation of dietary lipid or oil to the ration of ruminants [35]. Some of the possible mechanism by which added oil can reduce CH_4 emission include: (1) by increasing the amount of energy used to digest fiber (mostly in long chain fatty acids); (2) dry matter intake lowering (if total dietary lipid exceeds 6-7%); (3) via suppuration of methanogens mainly in medium-chain fatty acids; (4) through overpowering of rumen protozoa; and (5) to a) to a restricted extent via bio-hydrogenation [6, 35]. According to the evidence of some researchers, a 1% increase of dietary fat can reduce enteric methane emission between 4 - 5% [32, 35].

Grinding grain feed or physical processing of grain feed aimed to improve its digestibility is expected to decrease enteric methane emission intensity [15]. Improving quality of diet also result in better animal production performance as well as decreasing methane production in the rumen as measured by decrease in methane emission per unit of animal product [6].

Strategic supplementation of the diet like chemical treatment of low quality feeds or pasture, ration balancing and crop selection for straw quality are effective mitigation strategies, but these technology has been poorly practiced in animal feeding [15].

Dietary Protein management is also a good strategy to reduce methane emission. An increase of protein content of diet or ration can also improve digestibility and reduce overall methane emission per unit of animal product [36].

(3) Husbandry Management

Methane emission from a given farm depends on the number of animals and the emission per head [24]. Increasing an individual animal productivity can be a very effective strategy for decreasing GHG emission pee unit of animal product. Reduction of herd size is a good strategy, this would also increase feed availability and productivity of individual animals and the total herd, thus sinking methane emission intensity [15].

Minimizing disease and environmental stressor via an

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effective disease causing agent management strategy will improve productivity of the herd and results in reduction of CH_4 emission per unit of animal product as well as in overall herd of the farm [24].

Regarding the age of calves to reach slaughter weight and the number of days the cattle remained on feed in the feedlot to finish weight has effect on the rate of methane emission per animals. To resolve these problem improving animal nutrition and genetics can have a significant impact on GHG emission in beef and other meat animal production system [15]. In case of dairy farming, extending lactation period is the main strategy to reduce methane emission because it reduces herd energy demand and replacement rate [12].

(4) Chemical Additives

Some chemicals are used in animal feed for the sake of improving feed digestibility. Recently it is known that some chemical agents such as ionophores (monensin), unsaturated fatty acid, sulphate, nitrate, fumarate and halogenated methane analogues (Bromochloromethane (BCM)) are able to reduce methane production from ruminant animals [16,25,37].

Adding nitrate to the ration result in reduced amount of CH_4 emission because it is converted to ammonium (NH_4^+) which leaves less H_2 available for methane production. This method may have applicability in place such as Australia and Brazil where nitrate could replace the urea which is added to low quality ration to nutritive value [10].

Bromochromomethane (BCM) is one of the most effective inhibitors and apparently reduce CH4 production by interfering with the Cobamide dependent methyl transferase step of methanogenesis [38]. Bromochromomethane (BCM) complexed in cyclodextrin CD; BCM-CD) results in the stained inhibition of CH4 production when fed to ruminants [39]. Moreover, an in vitro continuous fermentation system simulating rumen fermentation demonstrated that BCM significantly reduced methane production by (85-90%) and eliminated most methanogens, whereas there was no effect on total production, true digestibility of feed and of feed efficiency of microbial protein synthesis [40].

(5) Probiotic Supplements

There are some microbial feed additives that have been developed to improve productivity by directly influencing rumen fermentation [41]. Probiotics or direct fed microbial are used in the diet of ruminants to improve the health status, rumen fermentation and ultimately the animal per formance that could also reduce methane emission [42]. [43] Reported the use of probiotics in mitigation of methane from ruminants. Probiotics improved productivity by 7 to 8 percent resulting in reduced CH_4 per unit of product in cattle.

3.1.2 Methane Mitigation Targeting Manure Management

The most mitigation alternatives for greenhouse gas emission from stored manure, such as reducing the time of aeration, manure storage and stacking are generally aimed at reducing the time of allowed for microbial fermentation process to occur before land application. This kind of mitigation practices are more effective, but their economic feasibility is uncertain [15].

Table 1. Methane mitigation strategy from manure.

Slurry manur storage	Solid Manure Storage
Storage temperature	Prevent CH_4 formation
Manure acidification	Prevent anaerobic conditions
Reduced storage time	Reduced storage time
Prevent and repair leakage	Composting
Improve anaerobic digestion	Reduce manure moisture
Collect and combust methane	Storage temperature
Cover manure storage	Manure acidification

Source: [15]

3.2 Mitigating Emissions of Nitrous Oxide

Some of the strategies used for increasing the efficiency of N-Cycle in livestock production system and soil aeration should also lead to reduced N_2O emission [4].

Diminishing total ration protein contain and supplementing the ration with synthetic amino acid is an effective means of ammonia and N2O mitigation strategies for non-ruminants. Ammonia emission from liquid animal waste or slurry receiving the tannin supplemented diet was 8-49% lower than the control slurry. Tannin also lower ammonia emission by 20% when directly applied to the barn flor and 27%bafter a tannin excreta was applied to the soil [44]. In contrary to the economic value of the manure, tannin use can reduce N-release rate from manure and thus affect manure -N availability for plant growth [15].

Salt similarly has some mitigation effect of methane in animal production. Adding salt increase water intake in ruminants, this may force the animals both decreasing urinary nitrogen concetration and encouraging more frequent urination events thus spreading urine more evenly across grazing pasture [6].

Another mitigation strategy is by use of chemicals that inhibit the oxidation of ammonium to nitrate in soil and thereby reducing N_2O emission from urine [15]. Some of Nitrification inhibitors like (Dicyandiamide or 3,4-dimethylpyrazole phosphate) applied with slurry under simulat-

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ed Portuguese condition were very efficient in reducing nitrous oxide emission [45].

4. Conclusion

The livestock sector contribute indirectly and directly to greenhouse gas emission. Indirect emission include emission resulting from feed crops, farm operation, manure application, transportation, animal product processing and land use allocation for animal production while direct emission from livestock sources refers to enteric fermentation, excretions and respiration. Greenhouse gases most often associated with animal production are methane, nitrous oxide and carbon dioxide. Around 44% of animal emission are in the form of CH4 while N_2O represent 29% and CO_2 represent 27%. Livestock contribute to global GHG emission approximately 14.5%. Limiting emissions from livestock, without cooperating food security is an important effort to GHG emission. The main option for reducing GHG emission per unit of livestock production include: mitigating emissions of CH_4 through reducing methane emission from enteric fermentation through improved feed efficiency of individual animal, husbandry as well as genetic management and improved management of biogas and manure. The other one is mitigation emission of N_2O via more efficient use of nitrogenous fertilizer, proper manure feed management and by using different feed additives.

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