BOOKLET

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Online J. Anim. Feed Res., 14 (2): 86-164; March 25, 2024

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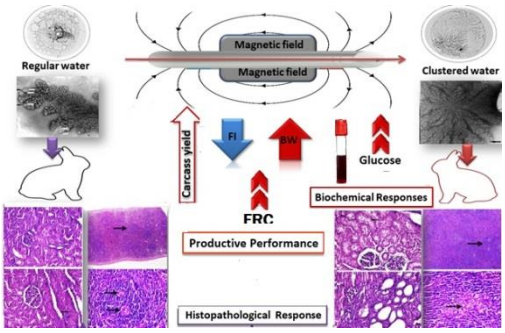
Volume 14 (2); March 25, 2024

Research Paper

Drinking magnetized water alters blood constituents, and structure of spleen and kidney in rabbits

El Sabry MI, Hassan A, Ebeid TA, Abou-Hashim F.
Online J. Anim. Feed Res., 14(2): 86-94, 2024; pii: S222877012400011-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.11

Abstract
This study aimed to investigate the effect of magnetic field on physicochemical properties of water and evaluating the effects of the magnetized water (MW) on the productive performance, liver enzymes, spleen and kidney structure of rabbits. Water samples were collected to determine pH and electrical conductivity (EC), and water structure was investigated by Transmission Electron Microscopy (TEM). Twenty-four weaned Rex rabbits, 21 d old, were allotted into two experimental groups: the first group was assigned as control, received regular tap water (TW), and the treated group, received MW, for 5 weeks. Productive traits were recorded weekly and at the end of the experiment, blood samples, spleen and kidney were collected for examinations. Results showed that pH and EC of MW were higher than those of TW. In addition, the arrangement MW cluster showed a unique alteration on the nano-scale. Growth performance indicators were similar in both experimental treatments, except FCR of the MW rabbits was better than that of the TW rabbits. The MW had no significant effect on plasma concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine in growing Rex rabbits received MW in comparison with those received regular TW. Spleen histological structure of rabbits of both groups was normal. However, the epithelial cells lining renal tubules of kidneys of rabbits in MW group appeared large with basophilic nuclei in comparison with TW group. Conclusively, magnetic field alters the physicochemical properties of water. The MW may consequently increase blood glucose level and spleen weight % and enhance kidney structure in growing rabbits. Moreover, more studies are still needed to know the benefits of providing the magnetized water to animals.
Keywords: Body weight; Liver; Magnetized water; Native water; Rabbits; Water quality.



[Full text-PDF]

Research Paper

Enhancing growth and milk production of dairy buffaloes through home-grown forages and complete nutrient diet

Aquino DL, Palacpac EP, Molina AM, Lacanilao CC, Garcia NP, Del Barrio AN, Fujihara T.
Online J. Anim. Feed Res., 14(2): 95-106, 2024; pii: S222877012400012-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.12

Abstract
This study aimed to boost the growth and milk production of dairy buffaloes while increasing farmers' income in Nueva Ecija, Philippines. The approach involved utilizing home-grown forages (HGF), such as napier grass and various legumes (*Leucaena, Rensonii, and Indigofera*), along with a complete nutrient diet (CND). For growing buffaloes, the CND consisted of 23 kg chopped napier grasses, 3 kg legumes, and 1 kg grower concentrates. For lactating buffaloes, the CND comprised 45 kg napier grass, 5 kg legumes, and 2 kg dairy concentrates. Sixty farmers, collectively raising 348 buffaloes, were trained in HGF production and CND preparation. In a 120-day feeding trial for growing buffaloes, a subset of 20 farmers participated, with 10 feeding their buffaloes CND and the other 10 serving as controls. The control group employed traditional feeding practices, involving tethered grazing on native pasture supplemented by cut-and-carry feeding of mixed native grasses. Implementing CND for growing buffaloes resulted in an average daily gain (ADG) of 0.46 kg or an improvement of 53.33% compared to the 0.30 kg ADG observed with traditional feeding. Moreover, CND implementation reduced feed costs, leading to a 98.54% increase in income per growing animal. In a separate feeding trial for lactating buffaloes, another subset of 20 farmers participated, with 10 feeding CND and the remaining 10 serving as controls (traditional feeding). Feeding CND to lactating buffaloes increased daily milk yield from 4.6 kg to 6.0 kg per animal, reflecting a 30.43% improvement compared to those fed with the control diet. This translated to a 41.31% increase in farmers' income over a 180-day lactation period. The findings underline the effectiveness of HGF production and CND

feeding in improving the performance of dairy buffaloes and increasing the financial well-being of farmers in Nueva Ecija, marking a significant advancement in sustainable dairy farming practices.
Keywords: Dairy buffaloes; Diet; Home-grown forages; Lactating period, Legumes.

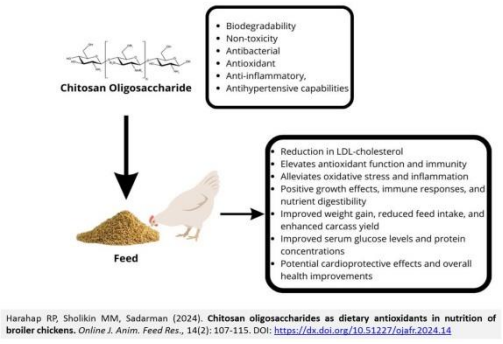
[Full text-PDF]

Review

Chitosan oligosaccharides as dietary antioxidants in nutrition of broiler chickens

Harahap RP, Sholikin MM, Sadarman.
Online J. Anim. Feed Res., 14(2): 107-115, 2024; pii: S222877012400013-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.13

Abstract
Chitosan oligosaccharides (COS) have attracted considerable attention in poultry research due to their diverse biological activities and possible effects on the welfare and productivity of broiler chickens. A thorough examination of many studies indicates that the influence of COS on indices such as antioxidative functions, growth performance, immunological responses, and metabolic implications in broilers is significant. For example, specific dosage levels of COS have significantly enhanced antioxidant activity, regulation of cholesterol levels, and improved growth performance. The research findings have provided evidence for COS's antioxidative and anti-inflammatory properties and its capacity to mitigate the effects caused by stress. Nevertheless, the effectiveness of reactions might be influenced by the dosage and may demonstrate variances. Broiler chickens are suggested to consume dietary COS levels between 350 mg/kg and 500 mg/kg feed to obtain antioxidant and immunological advantages. Nevertheless, the most favorable results regarding growth and the ability to absorb nutrients are typically found when the intake ranges from 0.5 g/kg to 1.0 g/kg feed. For yellow-feather broilers experiencing heat stress, it is advisable to administer a dosage of 200 mg/kg feed of COS. However, it is essential to closely observe dosages exceeding 2.5 g/kg since they may significantly impair growth performance. The diverse research on using COS in broiler management has provided valuable insights into its intricate nature. This review has highlighted the potential benefits of COS in enhancing chicken health and nutrition. However, it has also underscored the need for additional research to optimize its effectiveness in broiler production fully. It can be concluded that dietary COS in broiler chickens in doses ranging from 200 mg to 1000 mg/kg feed has a positive effect on growth performance, antioxidative properties, regulation of lipid metabolism, ability to mitigate stress, impact on meat quality, and carcass traits, but exceeding 2.5 g/kg feed may significantly impair in growth performance in the broiler.
Keywords: Antioxidative, Anti-inflammatory, Broiler, Chitosan Oligosaccharides, Growth Performance, Meat Quality.



Harahap RP, Sholikin MM, Sadarman (2024). **Chitosan oligosaccharides as dietary antioxidants in nutrition of broiler chickens.** *Online J. Anim. Feed Res.*, 14(2): 107-115. DOI: https://dx.doi.org/10.51227/ojafr.2024.14

[Full text-PDF]

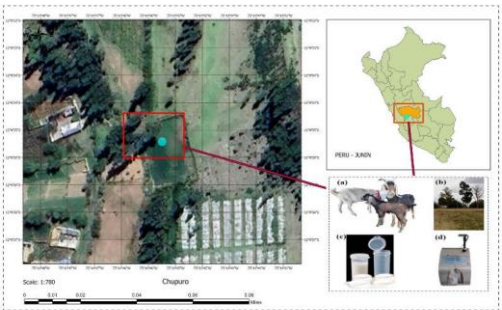
Research Paper

Physicochemical composition of Criolla and Criolla x Saanen goat milk according to age and parity in the central highlands of Peru

Garcia-Olarte E, Carhuas JN, Guillen MAF, Tacza AA, and Ramos EER.

Online J. Anim. Feed Res., 14(2): 116-123, 2024; pii: S222877012400014-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.14

Abstract
Present study aimed to analyze the physicochemical composition of goat milk based on the type of animal, age, and parity in Chupuro, Junin region in the country of Peru. A total of 24 goats were randomly selected, and divided into two groups: 12 native goats and 12 native goats crossed with Saanen. These groups (G1, G2, and G3) included three age categories (1.8, 2.5, and 3.2 years) and three parity levels (first, second, and third parity). The animals were monitored and fed considering their conversion and nutritional requirements. Before the morning milking, 100 ml of milk was extracted in sterile bottles, followed by agitation for 3 to 5 minutes, and the samples were transported using conservation and cooling techniques in a thermal box with ice cubes. These samples were analyzed in the special laboratory. Measurements of pH, acidity, density, lactose, total solids, fat, and protein were conducted using milk analyzer. The findings for the Criollo breed revealed a pH of 6.35 ± 0.31, a lactose concentration of 4.35%, total solids of 11.62 ± 1.31%, protein content of 4.12 ± 0.35%, and fat content of 3.40 ± 0.91%. In comparison, the Criollo x Saanen crossbreed exhibited a pH of 6.43 ± 0.13%, a lactose concentration of 4.45%, total solids of 12.63 ± 0.92%, protein content of 4.26 ± 0.28%, and fat content of 3.95 ± 0.69%. The results indicated that there were no significant differences in the types of milk from native goats and native goats crossed with Saanen. However, significant differences (P<0.05) were observed in density, lactose, total solids, fat,



Garcia-Olarte E, Carhuas JN, Guillen MAF, Tacza AA, and Ramos EER (2024). **Physicochemical composition of Criollo and Criollo x Saanen goat milk according to age and parity in the central highlands of Peru.** *Online J. Anim. Feed Res.*, 14(2): 116-123. DOI: https://dx.doi.org/10.51227/ojafr.2024.14

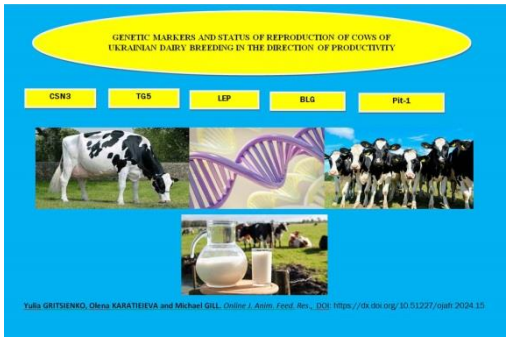
and protein between groups of different ages and parity levels. Crossbreeding with the Saanen breed is well received in the region, as it serves to improve milk production, with favorable percentages of fat, protein, lactose, and total solids.
Keywords: Milk quality, Native goats, Parity, Physicochemical composition, Saanen breed.

[Full text-PDF]

Research Paper

Identification of some genetic markers as productive and reproductive traits in Ukrainian dairy cattle breeding

Gritsienko Y, Karatieieva O and Gill M
Online J. Anim. Feed Res., 14(2): 124-136, 2024; pii: S222877012400015-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.15

Abstract


A Selection based on DNA markers is a breeding technique grounded in the genomic selection value of animals. The aim of the research is to study the genotypic profile of cows of different breeds in Ukrainian breeding in relation to the genes LEP, CSN3, TG5, BLG, and Pit-1 and to identify the probability of using them as markers for reproductive traits in cows. During the study, the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to analyze the genes and determine their polymorphic characteristics. The obtained data indicated that the same gene variants have varying effects in the studied breeds due to their diverse influences on the genomic background. Specifically, the homozygous state of leptin genes (LEPCC) and pituitary-specific transcription factor (PIT-1BB) was observed to impact the reproductive characteristics of cows in the evaluated breeds. Meanwhile, for the genes casein (CSN3AB), thyroglobulin (TG5CT), and beta-lactoglobulin (BLGAB), the heterozygous state of alleles was found to influence the key reproductive traits of dairy cattle of Holstein origin. The polymorphism of the genes CSN3, βLG, TG, PIT-1, and LEP indicated the presence of genetic potential for the reproductive function of cows and can be utilized as molecular markers in selective breeding, providing significant progress in improving not only the traits of dairy cattle productivity but also reproductive function. Therefore, in the implementation of selective breeding work, it is advisable to consider genotyping for the genes CSN3, βLG, TG, PIT-1, and LEP as an additional criterion for the selection of animals to enhance both their milk and reproductive characteristics.
Keywords: CSN3, Dairy cattle, Genetic potential, Marker genes, Polymorphism, Reproductive function.

[Full text-PDF]

Research Paper

Effectiveness of coconut meat waste in feed intake, digestion and protein retention in goats

Trung NB and Truong NB.
Online J. Anim. Feed Res., 14(2): 137-143, 2024; pii: S222877012400016-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.16

Abstract


The objective of the experiment was to determine the proportion of coconut meat waste on feed consumption, nutrient digestibility and nitrogen retention of goats. The experiment was conducted using a Latin square design on 4 male Bach Thao goat (16.2±2.93 kg). Treatments were 4 levels of coconut meat waste (CMW) at 0, 5, 10 and 15% in basal diet of rice distillers' by-product, cabbage waste, *Operculina turpethum* vines, urea and premix. Results indicated that dry matter intake per body weight tended to increase from CMW0 to CMW5 treatment but it was gradually reduced from CMW5 to CMW10 and CMW5 treatments (3.34; 3.50; 3.46 and 3.28, respectively). The ME consumption was higher at supplemented treatments coconut meat waste compared to CMW0 treatment. The nutrient digestibility (%) was gradually increased from CMW0 to CMW15 treatment. Similarly, digestive nutrients tended to increase with increasing coconut meat waste in the diet. The nitrogen retention was numerically lower for the CMW5, CMW10 and CMW15 treatments compared to CMW0, while daily weight gain was not different among treatments. In conclusion, 10% coconut meat waste could be used as an additional source of dietary regimen in goats, without negative effects on animal fattening performance.
Keywords: Agricultural waste, Alternative feedstuff, Digestibility, Local feeds, Small ruminants.

[Full text-PDF]

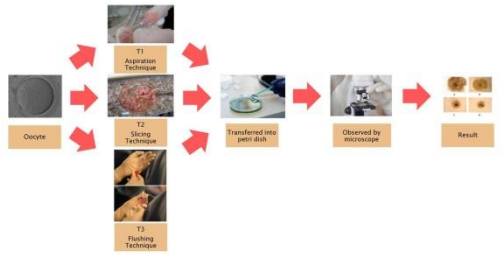
Research Paper

Aspiration, slicing, and flushing medium techniques in collecting oocytes of sheep: searching for the best method

Ondho YS, Sutiyono S, Setiatin ET, Sutopo S, Kurnianto E, Samsudewa D, Lestari DA, and Setiaji A.

Online J. Anim. Feed Res., 14(2): 144-149, 2024; pii: S222877012400017-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.17

Abstract
The aim of this study was to compare the effect of the techniques of aspiration, slicing, and flushing medium in collecting oocytes on the quantity and quality of oocytes, the average time used for collecting oocytes per ovary, and the volume of the medium used. The material utilized was 274 ovaries from ewes aged 2.5 to 3.5 years and body weight ranging between 25 and 35 kg. This study used a Completely Randomized Design consists of 3 treatments of techniques were aspiration, slicing, and flushing medium. The parameters measured included the average quantity, quality, and weight of oocytes per ovary (right/left), the effect of the techniques of aspiration, slicing, and flushing medium on the quantity and quality of oocytes, and the efficiency of use of medium and time spent to collect oocytes by using aspiration, slicing, and flushing medium techniques. Data were analyzed by one-way analysis of variance. The results showed that the aspiration technique collected the highest percentage ($P<0.01$) of oocytes quality A (38.49%) compared to the slicing technique (17.93%), and the flushing medium technique (11.71%). In terms of time, the aspiration technique was the fastest (8-10 minutes) compared to the slicing technique (10-12 minutes), and flushing medium technique (13-15 minutes); meanwhile, the aspiration technique was the most efficient technique (1-2 ml) compared to slicing technique (3-5 ml) and flushing medium technique (6-10 ml). In conclusion, the aspiration technique is the best one for oocyte collection from sheep ovaries. This technique proves to be efficient in terms of quantity and quality of the oocytes collected, time to perform, and medium to use.
Keywords: Ewes, Ovaries, Oocytes, Reproductive techniques, Sheep breeding.



Ondho YS, Sutiyono S, Setiatin ET, Sutopo S, Kurnianto E, Samsudewa D, Lestari DA, and Setiaji A (2024). **Aspiration, slicing, and flushing medium techniques in collecting oocytes of sheep: searching for the best method.** *Online J. Anim. Feed Res.*, 14(2): 144-149. DOI: https://dx.doi.org/10.51227/ojafr.2024.17

[Full text-PDF]

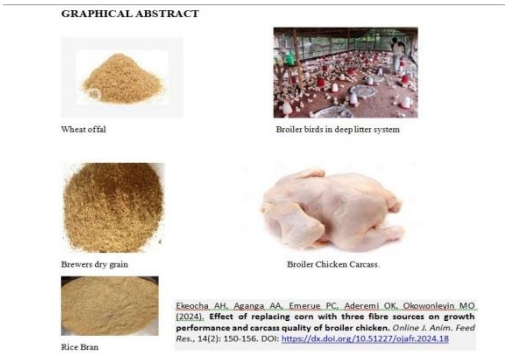
Research Paper

Effect of replacing corn with three fibre sources on growth performance and carcass quality of broiler chicken

Ekeocha AH, Aganga AA, Emerue PC, Aderemi OK, Okowonleyin MO.

Online J. Anim. Feed Res., 14(2): 150-156, 2024; pii: S222877012400018-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.18

Abstract
The experiment was carried out to evaluate the effect of replacing corn with three dietary fibre sources on the growth performance and carcass quality of broiler chicken. One hundred and forty four one-day old broiler chickens were used for the experiment. The birds were brooded for two weeks on commercial starter diet after which they were subjected to four experimental diets or treatments. The birds were randomly distributed into four treatments with three replicates, each replicate consisting of 12 birds. All birds in each treatment were fed with different diet and weighed at the end of every week. The experimental design used was a completely randomized design. The experimental treatments were designated as treatment T1, T2, T3, T4 while T1 was tagged as a control diet without any source of dietary fibre, T2 as a diet with wheat bran as a source of dietary fibre, T3 as a diet with rice bran as a source of dietary fibre, T4 as a diet with brewers dried grain (BDG) as a source of dietary fibre. There was no significant ($p>0.05$) difference in the initial weight of the birds across the treatments, but there was a significant ($p<0.05$) difference in the final weight and body weight gain of the birds where T2 had the highest body weight gain and T3 has the lowest body weight gain. This trend was also observed in the carcass. Based on the result of this experiment wheat bran can be used as a source of dietary fibre for better growth performance of broiler chicken at a low inclusion level.
Keywords: Broiler, Carcass, Corn, Dietary fibre, Growth performance.



[Full text-PDF]

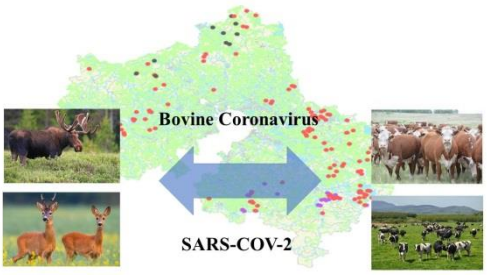
Research Paper

PCR-based study on viral pathogens circulation among cervids in the Moscow region

Yatsentyuk S, Krasnikova M, Dolinskaya K and Pchelnikov A.

Online J. Anim. Feed Res., 14(2): 157-164, 2024; pii: S222877012400019-14
DOI: https://dx.doi.org/10.51227/ojafr.2024.19

Abstract
A molecular survey of selected viruses in free-ranging cervids was conducted in 15 different districts of Moscow region. Samples were collected from 178 game animals including 144 moose (*Alces alces*), 19 roe deer (*Capreolus capreolus*) and 15 deer without species information. Nasal swabs and tissue samples including parts of the nasal septum, upper tracheal rings, lung, heart, liver, kidneys and pooled organ samples were tested using polymerase chain reaction (PCR). Samples were studied for pestiviruses, herpesviruses, coronaviruses, group A rotaviruses, adenoviruses, hepatitis e and parainfluenza type 3 virus. None of the samples were positive for Bovine Coronavirus and SARS-COV-2, hepatitis E virus and parainfluenza type 3 virus. PCR results were positive for bovine herpesviruses (5.05%), pestiviruses (0.56%), rotaviruses (1.68%). DNA of a new adenovirus, presumably causing a mild course of animal respiratory disease, was detected in samples of 6 animals (3.37%). In conclusion, the conducted studies have shown that game animals of the Moscow region can be a natural reservoir of cattle viruses, and this must be taken into account when planning and organizing measures for the control and eradication of such notifiable diseases as bovine viral diarrhoea and infectious bovine rhinotracheitis. Monitoring studies and general disease surveillance of wild animal populations provide additional information on the epidemiology of infectious diseases in the region and allow timely measures to be taken to protect wild animals, domestic animals and the public.



Keywords: Cattle, Deer, Epidemiology, PCR, Viral infection.

[Full text-PDF]

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DRINKING MAGNETIZED WATER ALTERS BLOOD CONSTITUENTS, AND STRUCTURE OF SPLEEN AND KIDNEY IN RABBITS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study aimed to investigate the effect of magnetic field on physicochemical properties of water and evaluating the effects of the magnetized water (MW) on the productive performance, liver enzymes, and histological structure of spleen and kidney of rabbits. Water samples were collected to determine pH and electrical conductivity (EC), and water structure was investigated by Transmission Electron Microscopy (TEM). Twenty-four weaned Rex rabbits, 21 d old, were allotted into two experimental groups: the first group was assigned as control, received regular tap water (TW), and the treated group, received MW, for 5 weeks. Productive traits were recorded weekly and at the end of the experiment, blood, spleen and kidney samples were collected for examinations. Results showed that pH and EC of MW were higher than those of TW. In addition, the arrangement MW cluster showed a unique alteration on the nano-scale. Growth performance indicators were similar in both experimental treatments, except FCR of the MW rabbits was better than that of the TW rabbits. The MW had no significant effect on plasma concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine in growing Rex rabbits received MW in comparison with those received regular TW. Spleen histological structure of rabbits of both groups was normal. However, the epithelial cells lining renal tubules of kidneys of rabbits in MW group appeared large with basophilic nuclei in comparison with TW group. Conclusively, magnetic field altered the physicochemical properties of water. The MW may consequently increase blood glucose level and spleen weight %, enhance kidney structure and improve FCR in growing rabbits. Moreover, more studies are still needed to know the benefits of providing the magnetized water to animals.

Keywords: Body weight; Liver; Magnetized water; Native water; Rabbits; Water quality.

INTRODUCTION

Many countries are facing critical challenges due to adverse impacts of climate change such water stress and the increase in temperature (El Sabry et al., 2023; Ebeid et al., 2023). Water is a vital nutrient in animal feeding. Thus, water quality, including the water content of minerals, could influence the performance of animals and the quality of their products (WHO, 1996; El Sabry et al., 2018; Aggrey et al., 2023).

Magnetized water (MW) application is exposing water to a magnetic field that alters the configuration of water clusters and the physical structure of water salts content (Alabi et al., 2015; Lingdinger 2021; El Sabry et al., 2022). In this context, Lingdinger (2021) suggested that the structured water (SW) cluster has a very structured and hexameric configuration, while the regular water cluster may have 3-dimensional shapes. Also, several studies reported that some physicochemical properties of water are affected by the magnetic field such as average size of water cluster, and induced changes in water pH, EC, and viscosity (Cheng and Weng, 2006; Cai et al. 2009; Alabi et al., 2015; El Sabry et al., 2018). However, the structuring mechanism is still poorly understood. Thus, MW/SW application has been suggested for improving water quality and mitigating salinity problems. It also has improved water quality and wellness, productivity, and reproductivity of animals (El-Hanoun et al., 2013; Zayed et al., 2018; El Sabry et al., 2021). It was reported that MW enhanced growth performance in growing rabbits (Helal et al., 2022), fertility in rabbit bucks (Attia et al., 2015), milk yield in dairy ewes (Shamsaldain and Al-Rawee, 2012), weight gain in geese (El-Hanoun et al., 2017) and egg production and hatchability in turkey (Ebrahim and Azab, 2017). Moreover, Zayed et al. (2018) found that MW had a protective effect on the kidney and maintained its structure in type-2 diabetic rats. On the other hand, other studies showed that MW did not affect the performance and livability of broiler chickens (Alhassani and Amin, 2012) and goats (Sargolzehi et al., 2009).

The abovementioned examples show a kind of contradiction about the effects of the MW application in farm animals' production. In addition, there is a lack of information about the influences of MW on the function and histological structure of organs. Therefore, this study aimed to study the effect of MW on the performance traits, liver and kidney functions, and the spleen and kidney structure in rabbits.

Citation: El Sabry MI, Hassan A, Ebeid TA, Abou-Hashim F (2024). Drinking magnetized water alters blood constituents, and structure of spleen and kidney in rabbits. *Online J. Anim. Feed Res.*, 14(2): 86-94. DOI: https://dx.doi.org/10.51227/ojafr.2024.11

MATERIALS AND METHODS

In the current study, twenty-four weaned Rex rabbits (three weeks old) of mixed sexes (1 male: 1 female) were used. In a semi-closed house, rabbits were kept individually in galvanized wire cages (40×50×35 cm^3) equipped with feeders and polyvinylchloride water lines. The ambient temperature ranged between 21°C and 26°C. The animals were randomly assigned into two experimental groups (12 rabbits per group): the control group, where rabbits received regular TW, and the treated group, where rabbits received MW. Before, starting the experiment, all rabbits were weighed individually to record the average of initial body weight of the rabbits of the control and treated groups, which were 370 ± 35 g and 368 ± 38 g, respectively. A standard growing diet was provided to rabbits *ad libitum* throughout the experimental period.

Strength and duration of water magnetization

The treated group (MW) received water was magnetized by exposing to 0.3 Tesla magnet apparatus (magnets obtained from K&J Magnetics, Inc., Pipersville, PA, USA) by fixing the magnet apparatus directly to the exterior surface of the polyvinylchloride water line through the experimental period to guarantee the exposure of fresh water to the magnetic field. The control group received regular tap water (Figure 1).

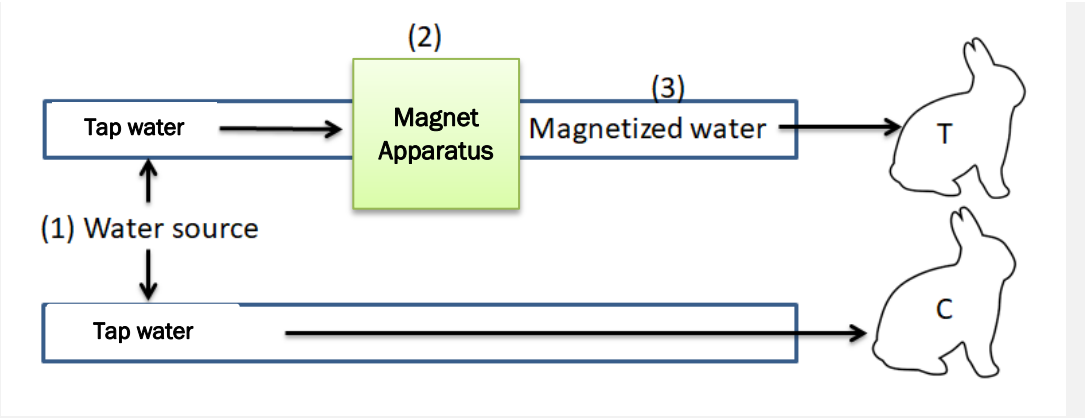

Figure 1 - The water supply for experimental animals showing magnetization process

Physicochemical properties of water

Three samples of water from both treatments were taken weekly to measure the key indicators of water structuring including pH using pH meter (805 MP, FISHER, Germany) and electric conductivity (EC) using a conductivity meter (mS m^{-1}) (WTW LF315 Conductivity Meter, UAS) at room temperature. After five weeks, water samples were also collected to determine the structure of clusters of water molecules using transmission electron microscope (JEOL JEM-1400, USA).

Blood biochemical parameters

Blood samples were collected from the jugular vein during slaughter bleeding into heparinized tubes for biochemical studies. Concentrations of blood plasma glucose (Glu), creatinine (CR), activities of alanine and aspartate aminotransferase (ALT, AST), and alkaline phosphatase (ALP) all were measured using colorimetric methods according to the instructions of the manufacturer.

Productive performance of rabbits

The feed intake and growth rate of rabbits were individually recorded weekly. By the end of the experiment at 56 day of age, rabbits were humanely sacrificed according to the Islamic method to determine the carcass composition. The relative organ weights as a percentage of the live body weight were also determined.

Spleen and kidney histological structure

For the histopathological investigation, five specimens from spleens and kidneys from both groups were taken and fixed in 10 % neutral formalin for 48 h. The tissues were dehydrated in graded concentrations of ethanol and cleared in xylene and then they were embedded in paraffin blocks. The tissues were cut into 5 µm thick sections and stained with hematoxylin and eosin. Five sections per group were examined using CX 31 light microscope (Olympus, Japan).

Statistical analysis

Data were analyzed using the SAS program (Version 9.4, 2013). A T-test was performed, where the magnetic field is the main effect on water pH and EC. Also, data of biochemical parameters and growth performance traits were analyzed using one-way analysis of variance. The following model was used:

$Yij = \mu + Li + eij$

Where: Yij: The j^{th} observation within the i^{th} line. μ: The overall mean. Li: The effect of the i^{th} type of water. Eij: Random error. Standard errors: SE. Means values were separated, when significance existed, using Duncan's multiple range test (Duncan's, 1955). Significance level was set at 5%.

RESULTS

Physicochemical properties of water

The pH value of the MW was higher than (7.20) the pH value of regular TW (6.82) ($P<0.01$). The mean of electrical conductivity (EC) of the MW420.3 mS.m-1 was greater than the EC of regular water 393.5 mS.m-1 ($P<0.01$). The transmission electron microscope of magnetized and regular water drops revealed the differences in the structure of water clusters on the nano-scale, which is suggested to be used as proof of the effect of the magnetic field on the water properties (Figures 2 and 3). The molecules of TW did not exhibit a distinct arrangement (Figure 2a). After passing water through the magnetic field, the molecules of the water droplet had a clear spherical shape border and the overview of the arrangement of the molecules appeared like a tree shape (Figure 2b). In Figure (3) edges of water clusters of magnetized and regular (un-magnetized) water clusters were different, being the edge of the MW cluster was well organized as well as the size of water molecules ranged from 9 to 12.7 nm (Figure 3b). While, the periphery of the TW cluster showed a lack of clear organization, with larger molecules measuring between 29.6 and 58.1 nm present (Figure 3a) (Figure 3a).

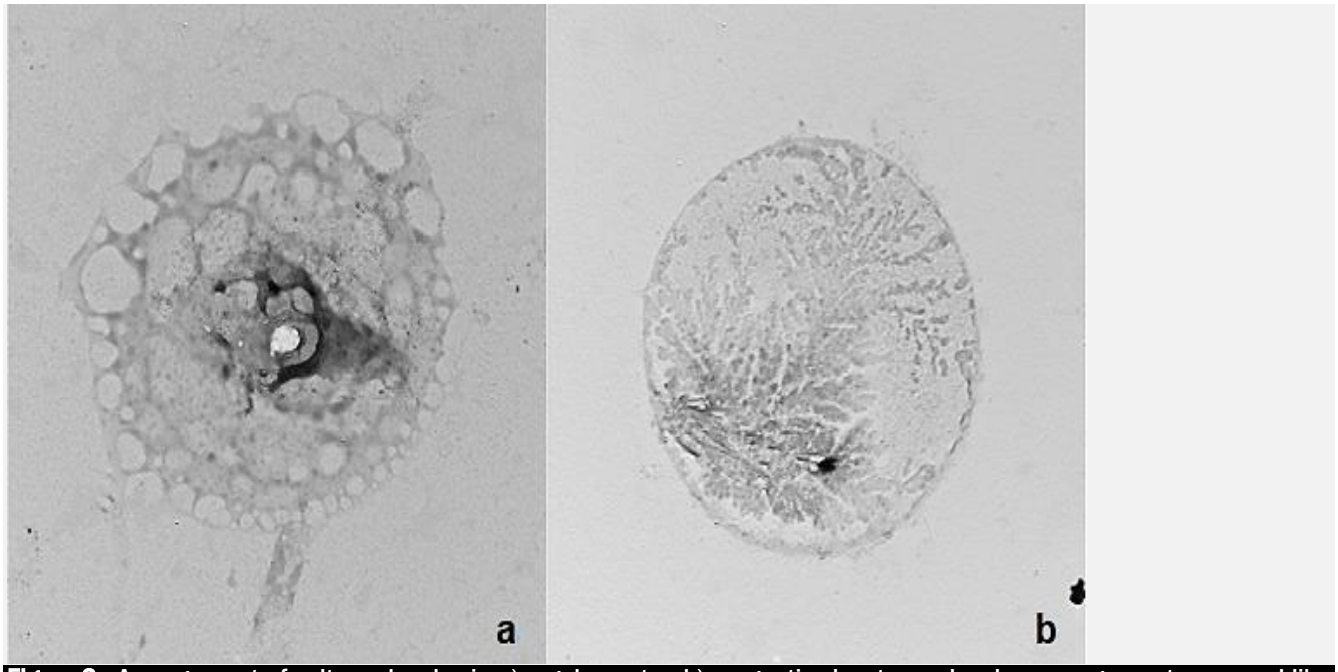

Figure 2 - Arrangement of salts molecules in a) regular water; b) magnetized water molecules arrangement appeared like a tree shape, using transmission electron microscope (20000X).

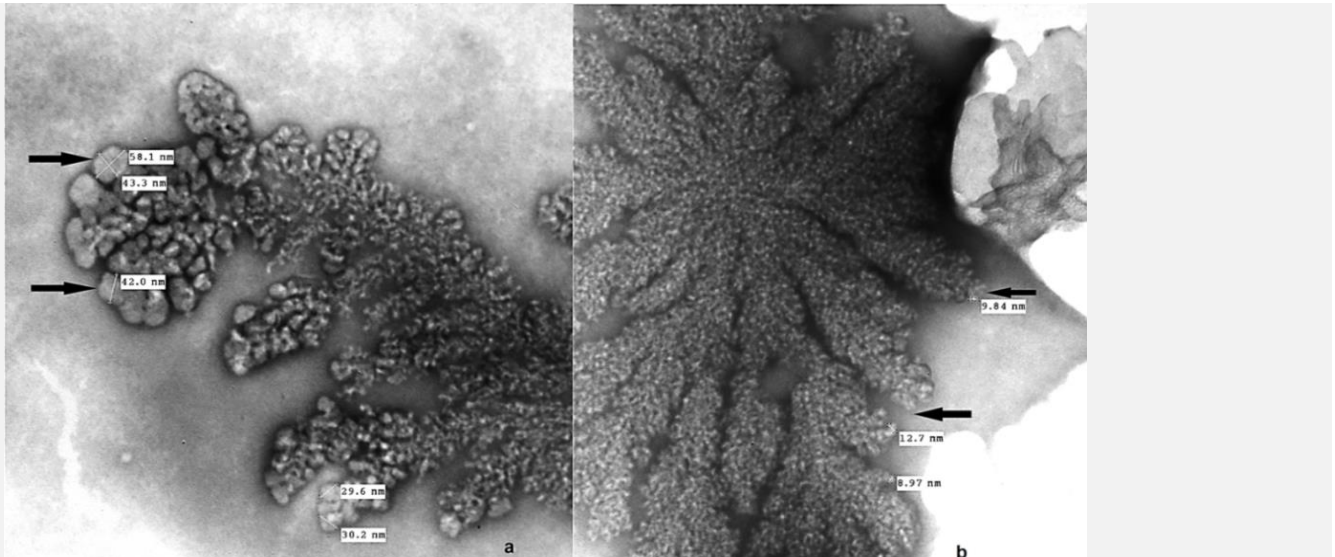

Figure 3 - Arrangement of water cluster molecules: a) regular water, a malformation at the edges of water clusters; b) magnetized water, molecules of water cluster are well structured (Transmission electron microscope, 120000X).

Blood biochemical parameters of growing Rex rabbits

Table 1 shows the biochemical response of growing Rex rabbits. No significant changes were found in plasma content of ALT, AST, ALP, and creatinine with a numerical increase in the group that received MW than those that received regular TW. Only glucose levels were significantly increased in groups that received MW ($P<0.05$).

Productive traits, carcass and organs relative weight

Throughout the experimental period, the growth performance traits of rabbits of MW or TW groups showed insignificant differences ($P>0.05$). At slaughter age (56 d of age), final body weight, feed intake, and fur weight of rabbits received either magnetized or regular drinking water were similar (Table 2). But, feed conversion ratio of the MW rabbits was better than that of the TW rabbits ($P=0.052$) .The relative weight of liver, spleen, and kidney of rabbits received either MW or regular TW were similar and within normal ranges (Table 3).

Table 1 - Biochemical parameters of rabbits received either regular (control) or magnetized water at 56 d of age

Parameter	Control	MW	P-Value
Glucose concentration (mg/dl)	$84.25^{a} \pm 2.61$	$94.44^{b} \pm 2.92$	<0.05
ALT (U/L)	2.50 ± 0.17	3.00 ± 0.18	NS
AST (U/L)	1.75 ± 0.20	1.38 ± 0.22	NS
Alkaline phosphatase (U/L)	68.25 ± 5.02	66.81 ± 5.61	NS
Creatinine (mg/dl)	0.60 ± 0.04	0.68 ± 0.04	NS

*No significant differences between treatments were observed; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 2 - Productive traits and relative weight of carcass and organs of rabbits received either regular (control) or magnetized drinking water (MW) at 56 d of age.

Rabbit productive traits	Control	MW	P-Value
Initial body weight (g)	370 ± 35	368 ± 38	0.70
Final body weight (g)	1584 ± 37	1677 ± 38	0.20
Total feed intake (g)	3551 ± 83	3507 ± 89	0.72
Feed Conversion Ratio	2.92 ± 0.05	2.67 ± 0.05	0.052

Means ± SE, within row, with different superscripts are significantly different. ($P<0.05$)

Table 3 - Carcass and organs weight % of rabbits received regular (control) or magnetized water at 56 d of age

Traits \ Water source	Control	Magnetized	P-value
Fasting weight (g)	1472	1629	NS
Empty carcass (g)	974	1004	NS
Carcass yield, %	60.7 ± 0.9	61.6 ± 0.8	NS
Spleen weight, g	1.04	1.07	NS
Heart weight, g	5.04	5.65	NS
Kidney weight, g	12.02	12.07	NS
liver weight, g	51.27	49.07	NS
Fur weight, g	148 ± 5	146 ± 5	NS
Relative weight of organs, %			
Liver	3.19 ± 0.16	3.08 ± 0.15	NS
Spleen	0.06 ± 0.004	0.06 ± 0.004	NS
Kidney	0.74 ± 0.026	0.73 ± 0.024	NS

*Means ± SE, within row, with different superscripts are significantly different. ($P<0.05$)

Alteration in spleen and kidney histological structure

The spleen histological structure of rabbits received TW showed normal histological structure with normal red and white pulp (Figure 3a,b). Similarly, spleen of rabbits received MW appeared normal in most examined sections with normal red and white pulp. But, in examined sections, the white pulp was slightly expanded with the increased number of lymphocytes and reticular cells. Kidneys of rabbits received TW showed normal renal tubules with normal epithelial lining and glomeruli (Figure 4a). On the other hand, kidney of rabbits received MW showed normal glomeruli and renal tubular epithelium, however, the epithelial cells lining some renal tubules appeared large with basophilic nuclei (Figure 4b).

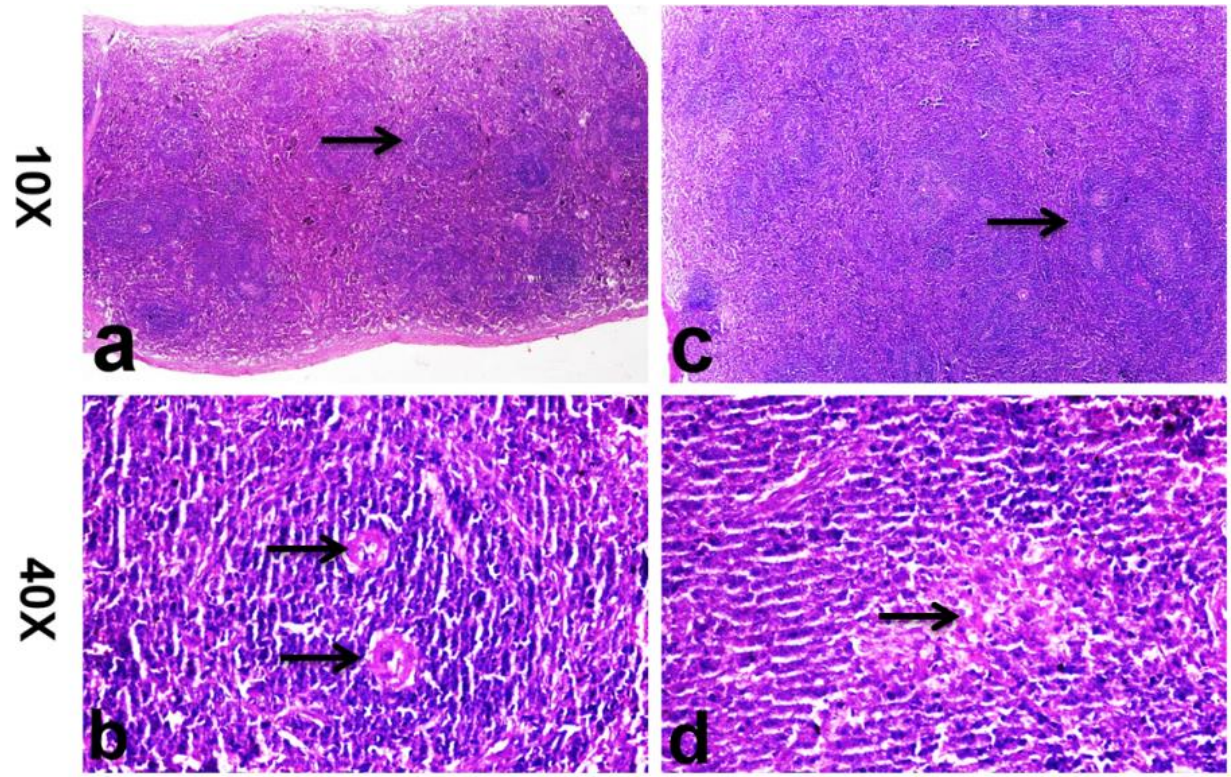

Figure 4 - Spleen (a and b) of rabbits received regular drinking water showing normal histological structure with normal red and white pulps (H&E,10X for a and H&E,40X for b). 4 c and d: spleen of rabbits received magnetized drinking water showing slightly expanded white pulp (H&E,10X for c) with increased number of reticular cells (H&E, 40X for d).

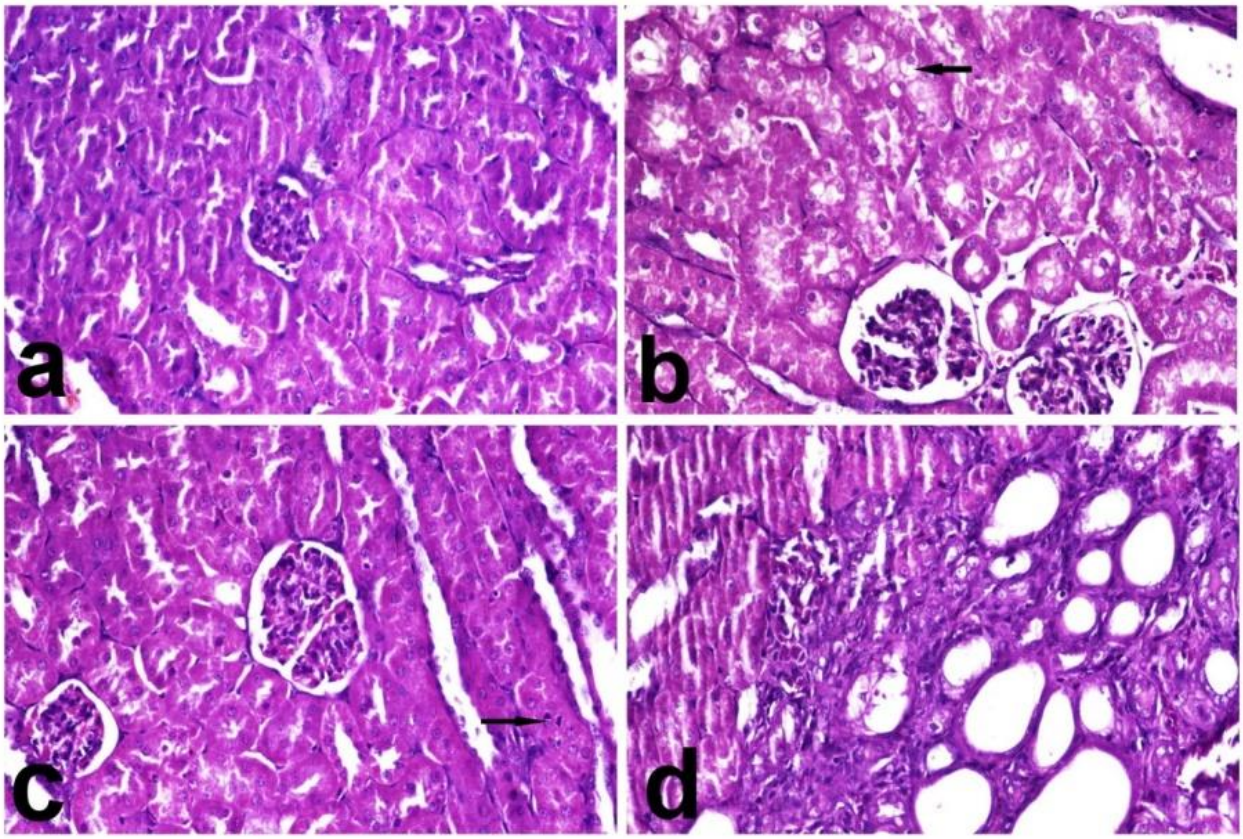

Figure 5 - Kidneys of (a) rabbits received normal drinking water showing normal renal tubules and glomeruli, (b, c & d) rabbits received MW showing mild degeneration of individual epithelial cells lining some renal tubules (arrows) (b), mitotic figure (arrow) (c) and presence of regenerative renal tubules characterized by basophilic cytoplasm and large vesicular nuclei (d), (H&E, 40X).

DISCUSSION

Physicochemical changes in water properties

Diamagnetic materials such as water and organic materials have a weak response to a magnetic field, which can be altered due to the magnetic field and the period of exposure to it (Alabi et al., 2015). When water is subjected to the magnetic field, the frequency of collision between ions was increased, and ions' configuration could be changed (Cai et al., 2009; Alabi et al., 2015). Moreover, Cho and Lee (2005), Xiao and Miwa (2017) mentioned that the magnetic treatment of water decreased water tension, altered magnetic spin H+, and weakened H-bonds. Subsequently, these alterations in chemical and physical properties of water are followed by more changes in the pH, electrical conductivity, and molecular energy of magnetically treated water (Parsons et al., 1997; Cai et al., 2009; Wang et al., 2018; El Sabry et al., 2018). Ebrahim and Azab (2017) suggested that treating water with a magnetic field improved the water molecule structure, which enhances the overall reactions of the MW. Although, the pH and EC values of MW were higher than those of TW, values were laid within the accepted pH value (6.5-8) and very satisfactory limits of EC (1.5- 5 ds.m-1) for poultry species (Ayers and Westcot, 1994; WHO, 1996). Also, Mahmoud, et al. (2015) elucidated that magnetic treatment induced greater effect on magnetic treated water in terms of pH, conductivity, salinity, and dissolved oxygen.

The transmission electron microscope was used to show if there are changes in the physicochemical properties of water due to exposure to the magnetic field. The current finding indicated changes in the physical feature of MW cultures at the nano-scale, which consequently might differently influence the digestive and absorptive processes in animals.

Changes in water properties might be attributed to one of the following explanations: 1) Cai et al. (2009) suggested that proton transfer in the closed hydrogen-bonded chains causes the magnetization of water, and 2) Xiao and Miwa (2017) mentioned that the magnetic treatment of water decreased water tension, altered magnetic spin H+, and possess weaker H-bonds. Therefore, it might be indicated that there is a potential to integrate magnet technology in manipulating some water properties, especially where water resources are limited. However, further studies are still needed for a better understanding the mode of action of magnetization on water.

Blood biochemical parameters

Water stress, high temperature, high feeding costs, and lack of proper management are the main reasons for the decline in the productivity of poultry and livestock (Szendrő et al., 2012; El Sabry et al., 2021; Abbas et al., 2022; El Sabry and Almasri, 2023). Under these circumstances, rabbits can be a potential source of animal protein due to their limited water requirement compared to livestock, ability to adapt to a variety of feed, and capacity to regulate their body temperature over a wide range of temperatures (Nistor et al., 2013; El Sabry et al., 2021).

As shown in Table 3, there is a numerical increase in plasma content of ALT, AST, ALP, and creatinine due to drinking of MW in comparison with TP. A mild increase in AST and ALT in the blood of rabbits received MW may show a positive effect on liver function. On the other hand, Helal et al. (2022) elucidated that MW reduced plasma concentrations of ALP, AST, ALT gamma-glutamyltransferase (GGT), total bilirubin, blood urea nitrogen and creatinine in growing rabbits.

The improvement in plasma glucose concentration might be attributed to enhancing the metabolism and nutrient transfer to various body cells. Rabbits that drank MW showed a significant increase in glucose concentration compared to those of the regular water group (Table 3). Similarly, Helal et al. (2022) postulated that MW increased plasma glucose concentration in growing rabbits. Also, Mahmoud et al. (2015) pointed out that administration of MA increased serum glucose concentration in growing black Balady rabbits. This improvement might be attributed to high conductivity MW, which may increase blood circulation and increase glucose uptake by the cells (Bonhomme-Faive et al., 1998; High et al., 2000; Alhammer et al., 2013).

Productive performance

Since water is the most important nutrient for farm animals, it is plausible that offering them MW may influence the productivity of the animal and/or its organ's characteristics. The current study's results showed no significant effect on the productive traits; final body weight, feed intake, fur weight, and carcass % through the experimental time. Nevertheless, it appears that all findings favored the MW group. These results could be due to providing the MW for a short period or the power of the magnet should be altered to achieve the desired effect on the studied traits. These results are in correspondence with Mahmoud et al. (2015) who demonstrated that drinking MW had no significant effect on feed intake, final body weight and weight gain in growing rabbits. In broiler chicks, similar findings were reported by Alhassani and Amin (2012), who found that there is no significant difference in the growth performance of broilers received MW or regular water.

In this study, better feed conversion ratio of MW group could be improved due to the new physiochemical properties of water that might enhance the availability of nutrients and the absorption process in rabbits. In this context, Helal et al. (2022) reported that average daily gain, growth rate and feed conversion ratio were elevated; however, feed intake was reduced due to drinking MW in comparison with non-magnetized water in growing rabbits. Also, El-Hanoun et al. (2013) found that the growth rate of offspring born to does that consumed MW during pregnancy had a greater growth rate than

those born to does that consumed regular tap water. This discrepancy in the results of growth performance might be due to the variation in physicochemical characteristics of MW and age and strain of rabbits.

Organs relative weight

Oliveira et al. (2013) and El Sabry et al. (2015) reported changes in organs' weight due to the exposure of the animal to improper environment or stress. For example, lighter spleen weight % was recorded due to exposing birds to uneven lighting programs (El Sabry et al., 2015). Since, rabbits' relative weight of both groups was within normal range, it could be speculated that the MW did not present a kind of stress on rabbits.

Alteration in spleen and kidney histological structures

The spleen is one of the main lymphoid organs that comprise two main compartments, which are white and red pulps. Functionally, white pulp plays an essential role in the immune response, while red pulp filters the blood from the old and damaged red blood cells (Mebius and Kraal, 2005; El Sabry et al., 2015). Therefore, it might be suggested that MW had no detrimental effect on the spleen histological structure. In this context, the slight expansion in white pulp might refer to an improvement in the immune status of rabbits that received MW. According to our knowledge, no studies in rabbits were conducted on the effect of MW on spleen histological structure. In rats, Al Saffar et al. (2013) indicated that rats treated with 250 gauss intensity of MW exhibited hyperplasia of the white pulp in their spleen tissue, whereas rats treated with 750 gauss intensity of MA displayed a notable hyperplasia of the lymphoid tissue in the periarterial sheath.

There are contradictions in the available information about the influence of MW on kidneys' histology due to the limited number of investigations on farm and laboratory animals. In addition, Khater and Ibraheim (2016) reported that water subjected to extremely high-power magnets (1.8 Tesla) could negatively affect the histological structure of the kidney in rats. Whereas, Zayed et al. (2018) found that the provision of MW (600 Gauss) protected the kidneys of type 2 diabetic rats from nephrotoxic damage by reducing hyperlipidemia, oxidative stress, uremia, and renal dysfunction. Ebrahim and Azab (2017) mentioned that MW could be a factor in breaking up kidney stones in humans. Sargolzehi et al. (2009) reported that MW did not influence urea and Na+, K+, Mg++, and P- in the blood of goats.

As shown in Fig (4a), kidneys of rabbits that received TW showed normal renal tubules with normal epithelial lining and glomeruli. These results are in harmony with Zayed et al. (2018) who noted that MW treatment prevented type 2 diabetic rat kidneys from nephrotoxic damage by reducing hyperlipidemia, uremia, oxidative stress, and renal dysfunction in rats. In the present study, mild hyperplasia of the renal tubular epithelium of some renal tubules in the kidney of rabbits that received MW may be due to the increase in the blood glucose level. Also, Thomson et al. (2001), Henegar et al. (2001) and Tobar et al. (2013) showed that high blood glucose level could induce glomerular enlargement is associated with hypertrophy of tubules. Moreover, enlargement of renal tubules is not necessarily a bad sign (Seely et al., 2018), and a few basophilic tubules are a normal feature in young rats (Frazier et al., 2012).

CONCLUSION

Water exposure to magnetic field alters physicochemical properties of water. The MW improves the FCR increases the relative weight of spleen. The positive changes in the liver and kidney structures of MW rabbits may indicate a positive influence on the animal's health. Moreover, more studies are still needed to determine the benefits of providing MW to animals.

DECLARATIONS

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Ethical Approval

This study was approved by the Institutional Animal Care and Use Committee of Cairo University, protocol No. (CU-II-F-37-17).

Author contribution

M.I. El Sabry, Fatma Abou-Hashim: paticpated in experimental design, collecting data, data analysis, and writing of the original manuscript. M.I. El Sabry, Tarek A. Ebeid: editing the final version. Azza Hassan: participated in histological investigation.

Data availability

Upon request.

Consent to participate
Consent was obtained from all authors.

Consent for publication
All participants have consented to the submission of the review article to the journal.

Competing interests
The authors declare no competing interests in this research and publication.

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ENHANCING GROWTH AND MILK PRODUCTION OF DAIRY BUFFALOES THROUGH HOME-GROWN FORAGES AND COMPLETE NUTRIENT DIET

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ABSTRACT: This study aimed to boost the growth and milk production of dairy buffaloes while increasing farmers' income in Nueva Ecija, Philippines. The approach involved utilizing home-grown forages (HGF), such as napier grass and various legumes (*Leucaena, Rensonii, and Indigofera*), along with a complete nutrient diet (CND). For growing buffaloes, the CND consisted of 23 kg chopped napier grasses, 3 kg legumes, and 1 kg grower concentrates. For lactating buffaloes, the CND comprised 45 kg napier grass, 5 kg legumes, and 2 kg dairy concentrates. Sixty farmers, collectively raising 348 buffaloes, were trained in HGF production and CND preparation. In a 120-day feeding trial for growing buffaloes, a subset of 20 farmers participated, with 10 feeding their buffaloes CND and the other 10 serving as controls. The control group employed traditional feeding practices, involving tethered grazing on native pasture supplemented by cut-and-carry feeding of mixed native grasses. Implementing CND for growing buffaloes resulted in an average daily gain (ADG) of 0.46 kg or an improvement of 53.33% compared to the 0.30 kg ADG observed with traditional feeding. Moreover, CND implementation reduced feed costs, leading to a 98.54% increase in income per growing animal. In a separate feeding trial for lactating buffaloes, another subset of 20 farmers participated, with 10 feeding CND and the remaining 10 serving as controls (traditional feeding). Feeding CND to lactating buffaloes increased daily milk yield from 4.6 kg to 6.0 kg per animal, reflecting a 30.43% improvement compared to those fed with the control diet. This translated to a 41.31% increase in farmers' income over a 180-day lactation period. The findings underline the effectiveness of HGF production and CND feeding in improving the performance of dairy buffaloes and increasing the financial well-being of farmers in Nueva Ecija, marking a significant advancement in sustainable dairy farming practices.

Keywords: Dairy buffaloes; Diet; Home-grown forages; Lactating period; Legumes.

INTRODUCTION

The demand for milk and milk products in the Philippines has steadily increased over the years. Local milk production only fulfills one percent of the total domestic requirements, necessitating substantial imports of dairy products (Hernandez et al., 2022). In 2020, the country imported a total volume of dairy products, equivalent to 2.936 million metric tons of liquid milk (LME) valued at US$1.08 billion (NDA, 2021). Projections indicate a significant rise in per capita consumption of meat, eggs, and milk products for the period 2015-2024, particularly in developing countries in Asia, including the Philippines (OECD/FAO, 2015). The increase in consumption is attributed to expected growth in per capita income in the region. Given the local demand for milk and milk products, government importation has become an inevitable strategy to meet the demand, underscoring the ongoing need to boost local milk production.

The government's farm mechanization program has partially displaced carabaos, the traditional source of draft power for crop farmers. However, through the efforts of the Department of Agriculture-Philippine Carabao Center (DA-PCC) and key stakeholders, carabaos have evolved into multipurpose animals, contributing significantly to protein-rich foods such as milk and meat.

Buffalo as a dairy animal

Dairy buffalo farming is gaining popularity in the Philippines (Tsuji, 2021). The Carabao Development Program (CDP), led by the DA-PCC, aims to enhance milk production from water buffaloes while simultaneously increasing the income and nutrition of smallholder farmers. The introduction of riverine buffaloes from Bulgaria, Brazil, and Italy, along with imported frozen semen from purebred dairy buffalo, aimed to jumpstart buffalo dairying and expedite the transformation of swamp buffalo (Philippine native carabao) into milk and meat producers (Cruz, 2012). Island-born and domestically reared dairy buffaloes can produce an average milk yield of 1,384 kg in a 287-day lactation, and in some cases, as high as 3,364.50 kg in a 300-day lactation (Aquino et al., 2017). With reported milk production figures and farm gate milk

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prices at PHP50/kg, dairy farmers can easily earn PHP168,225 per year, excluding the value of calf production (PCC, 2019). Of the country's 2.873 million carabaos in 2018, only 18,946 (0.659%) are considered potential dairy animals (PSA, 2020). In 2015, dairy carabaos contributed 7.12 metric tons of milk, representing 34.93% of the total national milk production of 20.38 metric tons (PSA, 2016). Records indicate that buffalo milk production consistently increased by 3%-6% annually, making it a significant contributor to the national milk supply.

Buffalo as a meat animal

Buffaloes globally contribute to over three million metric tons of buffalo meat products (Naveena and Kiran, 2014). The carabao's potential as a meat animal creates opportunities in the local wet market, particularly in processed meat products such as corned beef, hot dogs, and sausages. A study by Lapitan et al. (2007) showed that buffalo meat, known locally as "carabeef," from young and properly fed carabaos, is comparable to beef from cattle in terms of tenderness. Carabeef is recognized and favored by consumers as a healthy meat option due to its relatively low fat and cholesterol content, with a high proportion of lean meat (Kandeepan et al., 2009). Improving meat production from carabaos by developing practical rations to enhance average daily gain (ADG) and fattened weight is essential. As a meat animal, the carabao produced 144.680 metric tons of carabeef valued at PHP113.270 million in 2016 (PSA, 2016). This annual carabeef production from slaughtered buffaloes supports 78% of the country's total carabeef requirements.

Feeding management practices for buffaloes

Feeding buffaloes for both meat and milk production demands well-balanced rations to meet their nutritional needs for optimal growth and milk production. Meat and milk synthesis are among the most nutrient-demanding physiological and metabolic processes, and an imbalance in nutrients can lead to weight loss and health issues. A well-balanced ratio of protein, energy, vitamins, and minerals in a palatable feed is crucial for increasing milk production, live weight, as well as improving the health and fertility of the animals (Thomas, 2008).

Buffalo feeding is also influenced by seasonality of forage supply and cropping patterns. In regions like Nueva Ecija, forage is scarce during the dry season (December to May) and the rainy months (July to August) when all paddies are planted with rice (Aquino et al., 2020), resulting in poor growth, body condition, and milk production for buffaloes. To mitigate this, dairy farmers often use crop by-products like rice straw as fodder, but these have low nutritive value (Rusdy, 2022), leading to suboptimal animal performance and reduced income.

For dairy farmers, prioritizing forage quality is essential in optimizing the health and productivity of their buffalo herds. Understanding that the nutritional quality of forages significantly impacts animal productivity, farmers must focus on factors such as energy concentration and crude protein levels to meet the dietary requirements of dairy buffaloes (Collins et al., 2017). Forages with higher digestibility provide more energy per unit of dry matter consumed, thereby enhancing animal performance. Additionally, variations in forage composition, including fiber levels and susceptibility to microbial digestion, further underscore the importance of selecting high-quality forages. By prioritizing forage quality, farmers can optimize feed efficiency and promote the health and productivity of their buffalo herds, ensuring sustainable and profitable meat and milk production.

Home-grown forages and complete nutrient diet

Addressing the nutritional challenges faced by buffaloes in meat and milk production underscores the crucial role of home-grown forages (HGF) in ensuring a sustainable and productive feeding strategy. This importance aligns with the broader recognition of HGF's pivotal role in enhancing sustainability and productivity in ruminant production and dairying, as evidenced by insightful studies. For instance, Zucali et al. (2018) conducted a comprehensive environmental impact assessment of different cropping systems for home-grown feed in Northern Italy, emphasizing the crucial link between farmer choices in forage selection and the environmental footprint of milk production. Meanwhile, Chapman et al. (2014) explored the potential for increased HGF consumption and profit in non-irrigated dairy systems in southern Australia, highlighting the intricate balance between perennial ryegrass-dominant pastures and alternative forage options to achieve optimal productivity. Fariña and Chilibroste (2019) extended this perspective to Uruguay, analyzing farm systems to identify opportunities and challenges for the growth of pasture-based dairy production, underscoring the importance of overcoming economic, social, and environmental constraints. Additionally, Campbell (2019) delved into the utilization of HGF legumes, revealing their potential as protein sources for high-yielding dairy cows, particularly with the strategic use of tannins to enhance protein availability. Tharmaraj et al. (2014) further emphasized the practical implementation of complementary forages to achieve a substantial increase in forage harvested per hectare, showcasing the tangible benefits of diversified forage systems. Collectively, these studies underscore the significance of HGF in not only improving production efficiency and profitability but also in addressing environmental concerns and promoting sustainable practices in ruminant production and dairying.

Elevating sustainability and profitability, HGF lay the foundation for a comprehensive approach to modern livestock nutrition. The concept of providing ruminants or dairy animals with a complete and balanced diet is a critical aspect of contemporary livestock management, aiming to optimize production efficiency and overall health. This approach involves

formulating complete nutrient diets (CND), complete feeds, or total mixed rations (TMR) that encompass all necessary dietary components in appropriate proportions. Beigh et al. (2017) highlighted the importance of the complete feed system, emphasizing its role in preventing feed separation and stabilizing ruminal fermentation, ultimately leading to improved nutrient utilization. Delving into the comparative performance of pelleted napier grass-based TMR with *Indigofera*, Limao and Pomares (2022) showcased the potential of such complete ration mixes in terms of feed intake, weight gain, and rumen degradability. Additionally, Karunanayaka et al. (2022) underscored the significance of TMR in dairy cows, emphasizing its substantial effects on body weight, feed efficiency, milk yield, and reproductive performance. Collectively, these studies underscored the pivotal role of complete diets or feeds in ensuring a well-rounded and nutritionally balanced approach to ruminant and dairy animal nutrition, ultimately contributing to enhanced productivity and overall well-being.

Given the above context, we hypothesize that the integration of a sustainable supply of HGF with a well-formulated CND will significantly enhance the growth and milk production of dairy buffaloes in the province of Nueva Ecija. We posit that the strategic combination of HGF and CND will result in improved animal performance, optimal body weight, enhanced feed efficiency, increased milk yield, and improved cost-benefit outcomes. This hypothesis establishes the groundwork for a novel and comprehensive approach to ruminant and dairy animal nutrition in the Philippines, focusing on the synergy between locally sourced forages and carefully designed CND to achieve holistic benefits for the animals and increased economic viability for the farmers.

The current study aims to enhance the growth and milk production of dairy buffaloes in the province of Nueva Ecija in the Philippines by establishing a sustainable supply of HGF and developing a CND. Specifically, the objectives include establishing village-scale production of home-grown grasses and legumes, utilizing harvested HGF to develop and test a CND, and evaluating animal performance and the benefits of feeding the diet to growing and lactating buffaloes using a cost-benefit analysis.

MATERIALS AND METHODS

Preliminary survey

A preliminary survey was conducted to assess the current feeding management practices of dairy farmers in Nueva Ecija. The survey involved personal interviews with 168 dairy farmer-informants from various locations, including rain-fed and irrigated areas, as well as different herd sizes (less than 5 head, 6-10 head, 11-20 head, and more than 20 head).

Capability building

Prior to the technology demonstrations, partner farmers from Nueva Ecija participated in a training program titled "Science-Based Production of Grasses and Legumes for Year-Round Supply of Fodders". This training equipped the farmers with practical skills related to land preparation, preparation of planting materials, actual planting, and cultural management practices for their forage gardens. Additionally, the research project established a legume nursery and provided farmers with thousands of legume-potted seedlings. Napier grass cuttings and legume seedlings were distributed to the farmers based on a first-come-first-served basis upon completion of land preparation. Essential tools and farm equipment, including hand tractors and water pumps, were provided to support their farm demonstrations.

Activity 1: production of home-grown grasses and legumes

This activity encompassed two major components: the production of home-grown grass and legume plantations for seed and the production of HGF for use as a source of fodder.

Establishment of grass-legume plantation for seed production

A 10-hectare forage area at the DA-PCC site in Carranglan, Nueva Ecija, was designated for the establishment of grass and legume plantations for seed production. The grass-legume plantation included *Stylosanthes, Indigofera, Rensonii, Leucaena, Gliricidia,* and *Cajanus*, with each species occupying one hectare. Additionally, four hectares were allocated for improved grasses: Napier Pakchong (*Pennisetum purpureum* cv. Pakchong 1), Mulato (*Urochloa brizantha*), Mombasa (*Panicum maximum*), and Ruzi (*Brachiaria ruziziensis*).

Production of HGF

Sixty dairy farmers from 20 primary dairy cooperatives in Nueva Ecija participated in the on-farm production of HGF. These farmers were divided into two groups: 30 from irrigated areas (Group 1) and 30 from rain-fed areas (Group 2). Farmers in both groups owned one to five animals each (Table 1). The selection of partner farmers was based on their interest in participating in the research, their willingness to utilize their farm resources, and their commitment to record-keeping and data sharing.

Farmers were provided with four types of legume seedlings: *Indigofera, Rensonii, Leucaena,* and *Gliricidia*. However, Super Napier or Pakchong grass and two legume varieties, *Indigofera* and *Rensonii*, were the preferred choices for most of the farmers. The area of forage gardens was determined based on the number of animals raised and their annual fodder requirements. For example, an animal weighing 500 kg would require a daily feed equivalent to 2.5% of its body weight (on a dry matter basis), translating to a 550 m^2 forage garden per animal per year on a cut-and-carry system basis.

Table 1 - Layout of the production of home-grown forages

Forage Area	Animal holding (head)	Types of Forage	No. of Farmers
Group 1: Rain-fed	1-5	Grass and Legume	30
Group 2: Irrigated	1-5	Grass and Legume	30
Total			60

Grass includes mainly napier grass; Legumes include *Leucaena, Rensonii, and Indigofera*

Land preparation and planting method

Farmers conducted mechanized land preparation of their forage areas, including disc ploughing and harrowing to ensure proper soil preparation. Furrows were established for planting napier grass, while legumes were planted without furrows. Planting materials were distributed on a first-come-first-served basis, and planting was done in a systematic manner, alternating between napier grass and legumes.

Cultural management practices

Farmers implemented common management practices, including fertilizer application, irrigation, and off-baring of the plants. Fertilizer application was conducted two to three weeks after planting and one week after each harvest using a mixture of urea and complete fertilizer. Irrigation was performed as needed, with a focus on maintaining soil moisture during dry periods. Off-baring of napier grass was practiced twice a year to facilitate regrowth. Farmers monitored the growth and development of the plants closely.

Harvesting of forage and estimation of yield and quality

Forages were harvested manually by the partner farmers when the grass and legume plants reached maturity, generally around six months from planting. Harvesting intervals were set at 45-55 days for napier grass and 60 days for legumes. The fresh and dry matter yields per hectare were estimated using a quadrat method, and samples of grasses and legumes were collected and analyzed for nutrient composition at the DA-PCC Nutrition laboratory. The dry matter content was used to calculate the dry matter yield of napier grass and legumes per hectare.

Activity 2: development and testing of CND utilizing HGF for growing buffaloes

Development of CND for growing buffaloes

The development of the CND for growing buffaloes was based on feed reference standards published by Kearl (1982). The CND was designed to provide the necessary nutrients to support an average daily gain (ADG) of 500 grams in growing animals. It included a mixture of 23 kg chopped napier grasses and 3 kg legumes harvested from the HGF plus 1 kg grower concentrates and mineral mix.

Nutritional evaluation of CND

Twenty dairy farmers, each raising one growing buffalo heifer, participated in testing the CND. The heifers were selected based on similar breed (Bulgarian Murrah), initial weights, ages (around 1-2 years old), and body condition scores (2.5-3.0). Ten of the farmers fed their heifers with CND for 120 days. The other ten farmers served as the control group and followed traditional feeding practices, i.e., tethered grazing on native pasture plus 25 kg mixed native grasses offered through cut-and-carry feeding system. Animals in the control group also received I kg supplementary concentrates and mineral mix to balance their rations.

Development of CND for lactating buffaloes

The development of the CND for lactating buffaloes also followed feed reference standards by Kearl (1982). The CND was designed to provide the necessary nutrients to support a target milk production of seven kilograms of milk per day per cow. The composition included 45 kg of napier grass, 5 kg legumes, and 2 kg dairy concentrates, formulated to meet the nutrient requirements for daily milk production.

Actual CND feeding

Twenty dairy farmers participated in the feeding of CND using their own dairy animals, which were of the same breed (Bulgarian Murrah) and were in their third parity. These lactating buffaloes had an average initial daily milk yield of 4.43 kilograms. Ten farmers fed their lactating buffaloes with the developed CND, while the other ten followed traditional feeding practices, including tethered grazing and the cut-and-carry system. The parameters collected included daily feed intake, milk production, and changes in body weights of the animals. Simple cost-benefit analyses were also conducted, considering feed cost to produce a kilogram of milk and income from milk over feed cost.

RESULTS AND DISCUSSION

Preliminary survey on feeding practices

The preliminary survey revealed important insights into the feeding practices of dairy farmers in Nueva Ecija. Approximately 40% of the total farmer-informants reported having forage gardens ranging from 200-400 m^2, primarily planted with napier grass to serve as a source of fodder for their buffaloes. An alarming 92% of these farmers faced forage or feed scarcity, with 74.9% experiencing shortages from January to June and 17.2% from July to December. This scarcity partly explains why 76% of these farmers continued to follow traditional feeding practices, which involved tethering their animals in communal fields and providing minimal concentrate supplementation, with a focus on utilizing farm by-products.

Production of home-grown grasses and legumes

In this activity, the research project team played a crucial role in establishing a forage nursery and providing thousands of potted legume seedlings, including *Leucaena leucocephala, Indigofera suffruticosa, Desmodium rensonii,* and *Gliricidia sepium*. After receiving training on "Science and Technology-based Production of Grasses and Legumes for Year-round Fodder Supply for Dairy Buffaloes" and a "Refresher Course on Forage Production, Conservation, and Utilization", 175 farmers embarked on land preparation. The distribution of planting materials followed a first-come-first-served basis, considering the readiness of the planting area. Furthermore, the research project provided crucial farm equipment such as hand tractors and water pumps to support the farmers' efforts. A total of 60 dairy farmers with 348 buffaloes participated in this initiative. They received 129,620 legume seedlings and 613,000 Napier grass cuttings, enabling them to establish a collective 26.1 hectares of HGF. The project also supplied small farm implements, including sprinklers, water pumps, knapsack sprayers, and fertilizers, as inputs for HGF production.

Monitored growth rate of plants

Comparing irrigated and rain-fed areas, the growth and regrowth rates of napier grass, *Rensonii*, and *Indigofera* indicated faster development in the irrigated areas at both 60-day and 90-day harvest intervals, with plant heights of 183 cm, 174 cm, and 160 cm, respectively. In contrast, slower growth was observed in the rain-fed areas, particularly for *Leucaena*, which reached only 44 cm in height at a 60-day cutting interval and 94 cm at a 90-day cutting interval (Table 2). The current research findings, illustrating faster growth rates of forage species in irrigated areas compared to rain-fed areas, align with existing literature indicating the significant influence of water availability on forage growth (Kumar et al., 2022; Ren et al., 2021; Baath et al., 2020; Mendoza-Grimón et al., 2021), emphasizing the importance of water management in optimizing forage and legume production for livestock feed.

Herbage yield of the forages

Indigofera demonstrated higher dry matter (DM) yields compared to *Rensonii* and *Leucaena*, regardless of whether they were grown in rain-fed or irrigated conditions (Table 3). In the irrigated areas, *Indigofera* stood out with the highest DM yield of 10,120.68 kg/ha at a 90-day cutting interval, while *Leucaena* had the lowest DM yield of 3,337.51 kg/ha. This data highlighted the preference of dairy farmers for *Indigofera* in HGF production, given its ability to thrive in both rain-fed and irrigated conditions while providing higher protein content compared to *Rensonii* and *Leucaena*.

Overall, DM yields of napier grass and the three legume species were notably higher in the irrigated areas compared to those in the rain-fed areas. Napier grass DM yields at a 90-day cutting frequency in both areas indicated an average increase of 13.44% compared to 60-day cutting intervals. While *Leucaena* showed no significant difference in DM yields based on planting sites and cutting frequencies, *Rensonii* displayed significant variations in DM yields between 60-day and 90-day cutting intervals. Similarly, *Indigofera* exhibited significant differences in DM yields between 90-day and 60-day cutting intervals. These results resonate with Geren et al. (2020) and Bantihun et al. (2022), which suggested that longer cutting intervals promote greater biomass accumulation, potentially resulting in higher forage productivity. Although direct comparisons are lacking, the overall trend supports the inference that forage grasses and legumes may yield more dry matter with a 90-day cutting interval, highlighting the importance of cutting frequency in maximizing productivity (Dinsa and Yalew, 2022).

Nutrient composition

The nutrient composition analysis of napier grass and legume species revealed the quality of available fodders for developing the CND (Table 4). *Leucaena* demonstrated the highest crude protein (CP) content, reaching 21.57% at a 45-day cutting frequency, followed by *Indigofera* (21.31%) and *Rensonii* (18.26%). *Indigofera* consistently outperformed *Leucaena* and *Rensonii* in terms of CP content at various cutting intervals. This supports the earlier observation that *Indigofera* exhibited higher CP content than some other legume species (Syamsi et al., 2022). The data also confirmed that farmers preferred *Indigofera* due to its higher DM yields and superior protein content, which indicated its capacity to support more animals per hectare of forage plantation.

Table 2 - Average plant heights of home-grown forages

Site/Location	Cutting Interval (days)	Napier grass (cm)	*Indigofera* (cm)	*Rensonii* (cm)	*Leucaena* (cm)
Group 1: Rain-fed	60	139	83	85	44
	90	150	152	147	74
Group 2: Irrigated	60	165	90	93	50
	90	183	160	174	94

Table 3 - Dry matter yield/hectare of home-grown forages planted by the farmers

Site/Location	Cutting Interval (days)	Napier grass (kg)	*Indigofera* (kg)	*Rensonii* (kg)	*Leucaena* (kg)
Group 1: Rain-fed	60	12,700.00	3,958.95	2,610.29	2,078.90
	90	14,300.00	8,069.41	6,727.58	3,185.22
Group 2: Irrigated	60	13,300.00	5,254.70	6,044.99	2,234.00
	90	15,200.00	10,120.68	8,437.45	3,337.51

Table 4 - Nutrient composition of home-grown forages

Days	Grass/ Legumes	Herbage Yield/Plant (g)	DM (%)	Ash (%)	OM (%)	Crude Fat (%)	Crude Protein (%)	Fiber (%)	ADF (%)	NDF (%)
45	Napier	1,100.00	24.79	13.81	86.19	8.31	11.9	25.95	-	52.72
	Indigofera	561.49	24.63	10.36	89.64	2.51	21.31	18.66	25.04	35.82
	Rensonii	260.48	23.36	8.15	91.85	1.27	18.26	26.42	23	35.99
	Leucaena	188.89	32.49	8.05	91.95	2.55	21.57	18.29	18.78	35.57
60	Napier	1,270.00	25.76	14.13	85.6	8.15	10.15	32.66	-	58.08
	Indigofera	1,067.00	25.84	10.22	89.78	2.4	23.31	19.12	21.11	31.61
	Rensonii	946.33	24.12	8.43	91.57	1.12	18.67	27.68	21.07	34.98
	Leucaena	568.75	22.04	7.57	92.44	2.6	22.96	19.68	18.02	32.92
90	Napier	1,730.00	27.5	14.41	85.59	7.69	9.95	32.66	-	59.67
	Indigofera	3,087.50	23.14	10.17	89.83	3.75	23.24	15.26	23.2	31.92
	Rensonii	1,878.60	23.4	8.17	91.83	2.84	20.01	25.28	23.13	36.19
	Leucaena	803.33	23.79	7.97	92.03	4.34	21.13	20.24	18.32	35.62

DM=Dry Matter; OM=Organic Matter; ADF=Acid Detergent Fiber; NDF=Neutral Detergent Fiber

Development and testing of CND utilizing HGF for growing buffaloes

Effect of CND on growing buffaloes

The technology demonstration involving the feeding of CND to growing buffaloes spanned 120 days. The 10 farmers who adopted CND observed an average final body weight of 296.56 kg, representing an average daily gain (ADG) of 0.46 kg. In contrast, buffaloes fed with the control diet reached a final weight of 270.44 kg, with an ADG of only 0.30 kg (Table 5). Initially, there was no significant difference in the initial weights of buffaloes between the two feeding groups. However, by the end of the feeding period, buffaloes on the CND showed a trend towards higher final weights compared to those on the standard ration (Figure 1), although the difference was not statistically significant. Notably, buffaloes fed with the CND exhibited a significantly higher ADG and total weight gain compared to those on the standard ration, with p-values of 0.002, indicating highly significant differences. This suggests that the CND contributed to improved growth performance, resulting in faster weight gain and greater overall growth in the buffaloes over the feeding period.

Effects of CND on feed intake of growing buffaloes

Buffaloes fed with CND consumed 27 kg/hd/day on an as-fed basis, consisting of 23 kg of napier grass, 3 kg of legume, and 1 kg of concentrates. In comparison, animals fed with the control diet, which included 25 kg of napier grass and 1 kg of concentrate, consumed 26 kg/day. Buffaloes given CND achieved a daily dry matter intake (DMI) of 7.42 kg, equivalent to 2.6% of their body weight. This DMI was 1.16 kg higher than buffaloes on the control diet, which had a DMI of 7.17 kg/day, representing 2.52% of their body weight. These observed DMIs aligned with the published data by Kearl (1982), which reported DMI ranges of 2.2% to 2.9% of body weight and ADGs between 0.25 kg and 0.50 kg for growing buffaloes. Previous studies on the effects of forage legumes, such as those included in the CND, corroborate our findings by demonstrating the positive impact of these legumes on DMI and growth rate in ruminants (Durango et al., 2021; Maña et al., 2023). For instance, Durango et al. (2021) showed that forage legumes *like Leucaena leucocephala* can improve DMI and nitrogen retention in Zebu steers, leading to enhanced growth performance under tropical conditions. Furthermore, the study by Maña et al. (2023) demonstrated that the inclusion of legumes like *Indigofera tinctoria* in mixed swards can increase feed intake and improve growth performance in goats. These findings collectively support the notion that incorporating forage legumes into the diets of ruminants can positively influence DMI and growth rates, ultimately enhancing overall productivity.

Simple cost-benefit analysis of CND feeding in growing buffaloes

Farmers who adopted CND feeding for their growing buffaloes incurred a higher daily feed cost (FC) of PHP58 compared to PHP45 for the control diet (Table 6). However, when the FC per kilogram of weight gain was calculated, the CND-fed buffaloes exhibited significantly lower costs of PHP126.27 compared to PHP150.00 for those on the control diet. This reduction of PHP24.14 in FC per kilogram of weight gain in buffaloes fed with CND was attributed to their higher ADG, indicating that the additional nutrients provided by CND were efficiently utilized by the animals for lean meat production. Based on the total weight gain, the income over the cost of feeding CND to buffaloes reached PHP5,718.00, representing a 98.54% increase compared to the PHP2,880.00 for buffaloes fed with the control diet.

Development and evaluating of CND for lactating buffaloes

Feed intake of CND-fed lactating buffaloes

The introduction of CND did not significantly affect the daily feed intake of lactating dairy buffaloes. Buffaloes fed with the control diet consumed slightly more on an as-fed basis, with 47.00 kg compared to 45.31 kg per day for those on CND (Table 7).

Effect of CND feeding on Milk production

The adoption and feeding of CND resulted in a significant improvement in daily milk production (Figure 2). Dairy buffaloes given CND produced an average daily milk yield of 6.0 kg, while those following their usual feeding practices (control diet) yielded 4.6 kg/head/day. This translated to an increase of 1.4 kg in daily milk yield due to CND feeding, resulting in a higher total milk yield over a 180-day lactation period. These findings are consistent with previous literature, suggesting that incorporating legumes into the diet, as seen in the CND, enhances milk production in dairy animals (Mutimura et al., 2018; Gannuscio et al., 2022).

Simple cost-benefit analysis of CND feeding in lactating buffaloes

The daily cost of feeding CND was PHP101, which was PHP4.74 higher than the PHP96.25/day for the control diet. However, when expressed in terms of FC to produce a kilogram of milk, the trend reversed. Farmers spent only PHP16.83 per day to produce a kilogram of milk when using CND, compared to PHP20.92 for those on the control diet. Feeding CND to buffaloes generated a higher income of PHP57,420, compared to only PHP40,635 with the control diet during 180 milking days, representing a 41.31% increase in farmers' income.

Table 5 - Change in body weight of growing buffaloes fed with standard ration and complete nutrient diet

Parameters	Standard ration (control)		Complete nutrient diet		P-value
	Mean/SE (N=10)	SD	Mean/SE (N=10)	SD	
Initial weight, kg	234.44±11.64	34.92	241.44±12.72	38.16	0.909
Final weight, kg	270.44±12.48	37.44	296.56±17.77	53.32	0.083
ADG, kg	0.30±0.05	0.16	0.46±0.06	0.2	0.002**
Total weight gain, kg	36.00±6.31	18.93	55.12±07.88	23.63	0.002**

** highly significant (P<0.01); ADG = Average Daily Gain

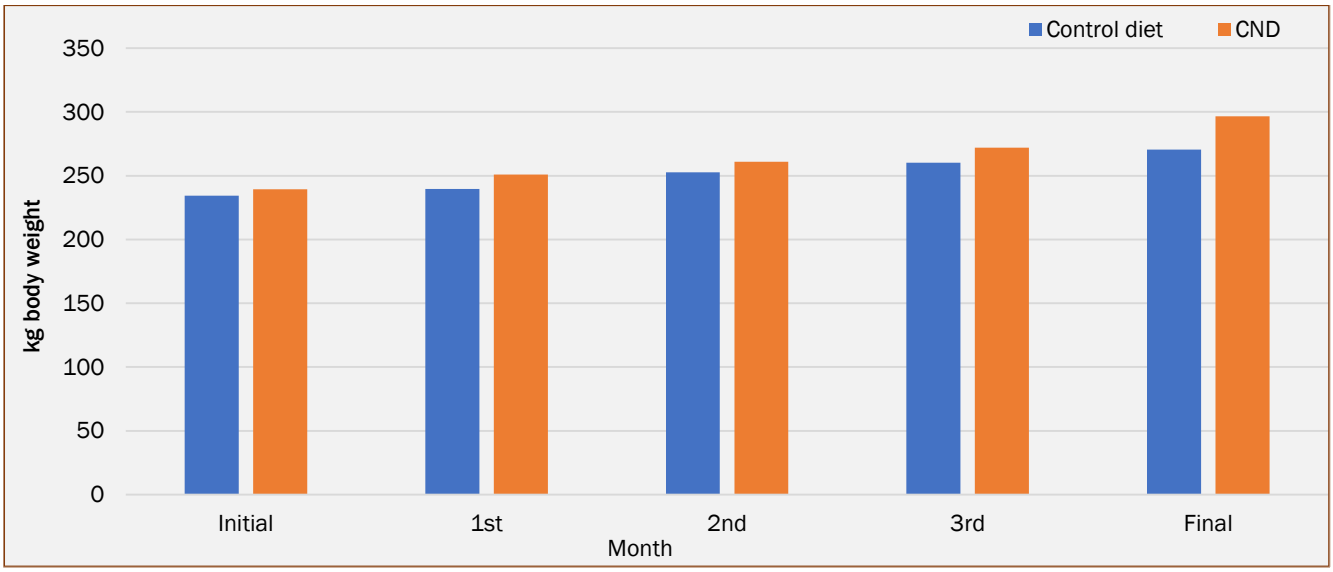

Figure 1 - Monthly body weight of buffaloes fed with complete nutrient diet (CND) and control diet

Table 6 - Simple cost-benefit analysis of feeding complete nutrient diet (CND) to growing buffaloes

Parameters	Standard ration (control)	Complete nutrient diet
No. of animals	10	10
Feeding period, days	120	120
Initial weight, kg	234.44	241.44
Final weight, kg	270.44	296.56
Total weight gain, kg (a)	36[a]	55.12[b]
Average daily gain, kg	0.30[a]	0.46[b]
Income from total weight gain, PHP*(b)	8,280	12,678
Feed intake, kg	26	27
Grass**	25	23
Legume***	–	3
Concentrate****	1	1
Feed cost/day, PHP	45	58
Feed cost/120 d, PHP (c)	5,400	6,960
Feed cost/kg BW, PHP (c/a)	150.00	126.27
Income-Feed Cost, PHP (b-c)	2,880	5,718
% Income Improvement		98.54

*PHP230/kg lean x total weight gain (kg); ** PHP1.00/kg; *** PHP 5/kg; **** PHP 20/kg; (PHP: Philippine peso)

Table 7 - Simple cost-benefit analysis of feeding complete nutrient diet to dairy buffaloes

Parameters	Standard ration (control)	Complete nutrient diet
No. of animals	10	10
Milk yield, kg/d	4.6a	6.0b
Total Milk Yield, kg **(a)**	828	1,080
Days in milk	180	180
Income from milk sales, PHP* (b)	57,960	75,600
Feed intake, kg/d	47	45.31
Feed cost/day, PHP	96.25	101
Feed cost/180 d, PHP (c)	17,325	18,180
Feed cost/kg milk, PHP (c/a)	20.92	16.83
Income-Feed Cost, PHP (b-c)	40,635	57,420
% Income Improvement	—	41.31

*PHP 70/kg

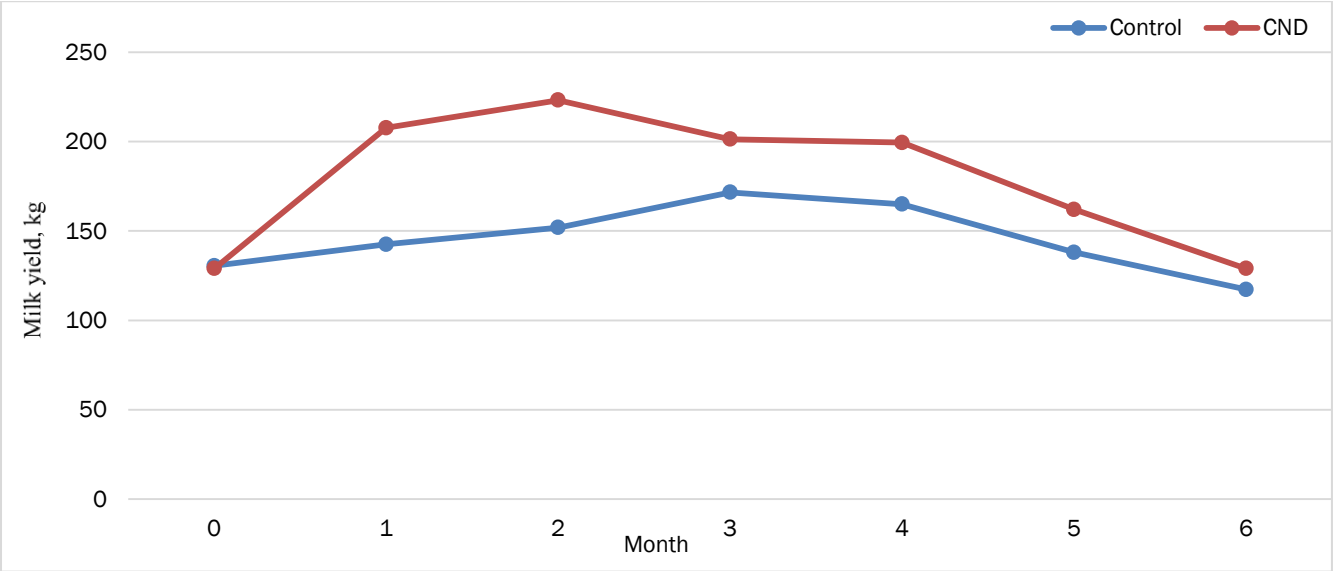

Figure 2 - Milk production of dairy buffaloes fed with complete nutrient diet (CND) and control diet

CONCLUSION

This study successfully addressed its objectives of enhancing the growth and milk production of dairy buffaloes in the province of Nueva Ecija, Philippines, through the strategic integration of home-grown forages (HGF) and a complete nutrient diet (CND). The findings align with the formulated hypothesis, as the synergistic combination of HGF and CND resulted in notable improvements in animal performance, including increased average daily gain in growing buffaloes and enhanced daily milk yield in lactating buffaloes. The results further demonstrated the economic viability of the approach, with a significant increase in income for farmers who adopted CND feeding practices. Despite the lack of statistical significance in some growth parameters, the observed positive trends in body weight, feed efficiency, and milk production support the overall success of the study. This research contributes to the advancement of sustainable dairy farming practices in the Philippines, emphasizing the importance of locally sourced forages and well-formulated CNDs in optimizing ruminant nutrition and promoting economic well-being among smallholder farmers.

DECLARATIONS

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Authors' contribution

The first author spearheaded the design and execution of the experiments, conducted results analysis, and contributed to the writing. The second author was involved in writing, results analysis, and refining the manuscript's structure. The third and fourth authors participated in data collection and lab analysis. The fifth author played a key role in establishing HGF production sites and coordinating with participating farmers. Lastly, the sixth and seventh authors contributed to the results analysis.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical considerations

The feeding trials conducted as part of this research were carried out in strict accordance with the ethical guidelines and protocols established by the Research Ethics Committee of the funding agency. The welfare and humane treatment of all animals involved in the trials were of paramount concern, and every effort was made to ensure their well-being throughout the experimental process. Additionally, the collection and handling of data from participating farmers adhered rigorously to the principles outlined in the Data Privacy Act of the Philippines. The confidentiality and privacy of farmer-related information were strictly maintained, and all data were handled with the utmost sensitivity and in compliance with relevant legal and ethical standards. Likewise, this study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations.

Competing interests

The authors declare no competing interests.

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CHITOSAN OLIGOSACCHARIDES AS DIETARY ANTIOXIDANTS IN NUTRITION OF BROILER CHICKENS: A REVIEW

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Chitosan oligosaccharides (COS) have attracted considerable attention in poultry research due to their diverse biological activities and possible effects on the welfare and productivity of broiler chickens. A thorough examination of many studies indicates that the influence of COS on indices such as antioxidative functions, growth performance, immunological responses, and metabolic implications in broilers is significant. For example, specific dosage levels of COS have significantly enhanced antioxidant activity, regulation of cholesterol levels, and improved growth performance. The research findings have provided evidence for COS's antioxidative and anti-inflammatory properties and its capacity to mitigate the effects caused by stress. Nevertheless, the effectiveness of reactions might be influenced by the dosage and may demonstrate variances. Broiler chickens are suggested to consume dietary COS levels between 350 mg/kg and 500 mg/kg feed to obtain antioxidant and immunological advantages. Nevertheless, the most favourable results regarding growth and the ability to absorb nutrients are typically found when the intake ranges from 0.5 g/kg to 1.0 g/kg feed. For yellow-feather broilers experiencing heat stress, it is advisable to administer a dosage of 200 mg/kg feed of COS. However, it is essential to closely observe dosages exceeding 2.5 g/kg since they may significantly impair growth performance. The diverse research on using COS in broiler management has provided valuable insights into its intricate nature. This review has highlighted the potential benefits of COS in enhancing chicken health and nutrition. However, it has also underscored the need for additional research to optimize its effectiveness entirely in broiler performance. It can be concluded that dietary COS in broiler chickens in doses ranging from 200 mg to 1000 mg/kg feed has a positive effect on growth performance, antioxidative properties, regulation of lipid metabolism, ability to mitigate stress, impact on meat quality, and carcass traits, but exceeding 2.5 g/kg feed may significantly impair in growth performance in the broiler chickens strain Cobb 500.

Keywords: Antioxidative, Anti-inflammatory, Broiler, Chitosan oligosaccharides, Growth Performance, Meat Quality.

INTRODUCTION

The production of broiler chickens is crucial in providing the worldwide need for protein of superior quality. Consequently, ensuring broiler chickens' well-being and efficiency is essential for the poultry sector. Nevertheless, broiler chickens frequently face many environmental stresses, such as exposure to low or high temperatures, potentially impacting their overall welfare and efficiency (Quinteiro-Filho et al., 2010). Throughout the context of physiological problems, two significant concerns that occur are oxidative stress and inflammation.

The occurrence of oxidative stress in broiler chickens is characterized by an imbalance between the release of reactive oxygen species (ROS) and the ability of the antioxidant defence system to neutralize them (Surai et al., 2019). This condition can lead to cellular harm, hindered growth, and diminished general well-being in these broiler chickens. Simultaneously, inflammation, though a protective response, can become detrimental when chronic, damaging tissue and compromising the immune system. Furthermore, enhancing the antioxidative functions, growth performance, immunological responses, and metabolic consequences are paramount due to their direct impact on poultry health and production efficacy.

Chitosan oligosaccharides (COS) are a natural polymer derived from chitin's deacetylation process (Lodhi et al., 2014). These chitosan oligosaccharides possess distinctive biochemical characteristics and exhibit minimal toxicity, making them a promising candidate for revolutionizing as feed additives (Elnesr et al., 2022; Uyanga et al., 2023). Recently, there has been a growing interest in using natural feed additives within the poultry sector, particularly in broiler performance. The research areas encompass antioxidative functions, growth performance, carcass characteristics

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response, anti-inflammatory activities, and effects on lipid metabolisms. Although significant insights have been gained from this research, a comprehensive comprehension of the role and consequences of chitosan oligosaccharides in broiler performance remains necessary. The objective of this study is to critically examine the existing studies to establish a comprehensive comprehension of the present state of research on this particular topic. Additionally, it intends to emphasize the areas that require future exploration to exploit the advantages of COS effectively. This evaluation provides a foundation for further exploration and potential adoption within the poultry industry. This review examined the diverse ramifications of chitosan oligosaccharides, encompassing their growth performance, antioxidative characteristics, control of lipid metabolism, stress mitigation capabilities, and influence on meat quality and carcass attributes.

Physical, chemical, and biological properties of chitosan oligosaccharides

Chitosan oligosaccharides (COS) are derived by deacetylation and depolymerization of chitin or chitosan, employing physical, chemical, or enzymatic techniques (Benchamas et al., 2021). Chitin is predominantly derived from the exoskeletons of crustaceans, such as shrimp and crabs, as well as the fungus cell walls. It is the second most abundant natural biopolymer, surpassed only by cellulose. Chitosan oligosaccharide comprises D-glucosamine and N-acetyl-D-glucosamine units connected by β-(1→4)-glycosidic linkages. Low molecular weight variants of chitosan possess a reduced molecular size and exhibit solubility in water, augmenting their bioavailability and efficacy in diverse applications (Naveed et al., 2019).

Chitosan oligosaccharides (COS) exhibit noteworthy biological characteristics, including antibacterial, antioxidant, anti-inflammatory, and antihypertensive capabilities (Guan et al., 2019). According to Lizardi-Mendoza et al. (2016), one of the pivotal attributes of COS is its biodegradability, positioning it as an environmentally friendly material by allowing it to decompose naturally, reducing concerns related to environmental accumulation and pollution. In addition to its eco-friendly nature, COS is recognized for its biocompatibility, implying that it is non-toxic to living cells, thus paving the way for its utilization in a spectrum of biomedical applications where cellular and tissue compatibility is paramount (Razi, 2022). This non-toxicity also extends its safe use to various other applications, ensuring that it does not threaten health or the environment (Lizardi-Mendoza et al., 2016). The water-soluble nature of COS enhances its bioavailability, facilitating its interaction and functionality in various applications, particularly in aqueous systems, which is often a prerequisite in industrial and biological applications.

Furthermore, the structural properties of chitosan oligosaccharides (COS) are noteworthy, as they cater to specific requirements across food, pharmaceutical, and biomedical sectors, providing stability, form, and function that can be tailored to meet the specific demands of various applications (Razi, 2022). The modified biomaterial exhibits a higher degree of deacetylation (DD), degree of polymerization (DP), reduced viscosity, and complete solubility in water, distinguishing it from its precursor biomaterials and other polysaccharides currently available (Naveed et al., 2019). The hydrophilic nature of this biopolymer has been skillfully harnessed in combination with other chemicals to effectively modify their surface structures, resulting in the creation of chemically appropriate surfaces (Prihandini et al., 2021). The degree of deacetylation of COS is usually less than approximately 50%, and their average molar mass is less than 10,000 Da. These properties contribute to their solubility in water and result in low-viscosity solutions (Shukla et al., 2015). According to the research conducted by Shukla et al. (2015), applying a COS coating on iron oxide nanoparticles reduces cellular damage and a modest generation of reactive oxygen species (ROS). Consequently, this coating effectively mitigates the cytotoxic effects of uncoated iron oxide nanoparticles.

Recent study of dietary chitosan oligosaccharides in broiler

The considerable influence of chitosan oligosaccharides (COS) in the diet of broiler chickens, as evidenced by various research, presents a persuasive account of its potential utility in improving the well-being and efficiency of poultry. The present study, as outlined in Table 1, comprehensively examines multiple investigations examining dietary COS effects on broilers. Each study has contributed valuable insights into different aspects and results associated with dietary COS in broilers. These studies, conducted over a range of years and with different study goals, investigate the effects of varying dietary COS on parameters such as antioxidative functions, growth performance, immunological responses, and metabolic consequences, among other factors. For example, the research conducted by Xiaocong et al. (2017) and Keser et al. (2012) investigates the antioxidative and biochemical profiles, uncovering significant results such as improved antioxidant activity and regulated cholesterol levels, respectively. Several research studies have also examined the intricate effects of COS on growth performance, with specific dose levels demonstrating the most favourable outcomes, as demonstrated by Osho and Adeola (2019) and Tufan and Arslan (2020).

Furthermore, the investigation into the antioxidative and anti-inflammatory effects, together with the ability to alleviate stress-induced effects, as demonstrated in the research conducted by Lan et al. (2023) and Fathi et al. (2023), presents a positive perspective on the function of chitosan oligosaccharides (COS) in the field of poultry nutrition and health. The various studies conducted on COS in broiler management highlight its complex character and provide a foundation for further investigation. It calls for a thorough examination to enhance our understanding and maximize the effectiveness of COS in broiler performance.

Table 1 - Summary of some reported studies on dietary chitosan oligosaccharides in broilers and its outcomes.

Level of COS	Parameters	Outcomes	Reference
0.025%	Performance and biochemical profiles in broiler such as serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, free fatty acids, total protein, urea, insulin, glucose, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT)	There were no significant effects of COS on the performance of broilers. It did result in significant reduction in LDL-cholesterol levels.	(Keser et al., 2012)
0, 200, 350 and 500 mg/kg feed	Antioxidative function, lymphocyte cycle and apoptosis of ileum mucosa in broiler	Dietary COS with 350 mg/kg and 500 mg/kg could improve the antioxidant function and accelerate lymphocyte proliferation but had no influence on lymphocyte apoptosis in the ileum mucosa of broilers.	(Xiaocong et al., 2017)
0, 0.5, 1.0, 1.5, 2.0 and 2.5 g of COS/kg feed	Growth performance, digestive functions, intestinal morphology, and immune organ	Level COS (between 0.5 g/kg and 1.0 g/kg) might be optimal for positive growth effects, immune responses and nutrient digestibility. However, they have also mentioned that growth performance showed signs of impairment at a higher level of 2.5 g/kg COS in the diet.	(Osho and Adeola, 2019)
0, 1 g/kg feed	Growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzyme	Dietary COS at 1 g/kg can mitigate stress and improve growth performance and immune function in broiler chickens, especially under dexamethasone (DEX-induced) stress conditions.	(Osho and Adeola, 2020)
0, 200 mg/kg feed	Growth performance, corticosterone, growth hormone, and insulin-like growth factor-1 concentration, relative organ weight, liver function, meat quality, muscle glycolytic metabolism and oxidative status	Dietary COS can be an effective feed additive for maintaining growth performance, liver function, meat quality, muscle glycolysis metabolism, and oxidative status in yellow-feather broilers under heat-stress conditions. Dietary COS could also alleviate heat stress, as evidenced by the improved growth performance and reduced heat stress markers in broilers.	(Chang et al., 2020)
0, 100, and 200 mg/kg feed	Oxidative stress and inflammation response in liver and spleen	Dietary COS at 200 mg/kg of diet significantly alleviated oxidative stress and inflammation response in yellow-feather broilers exposed to high ambient temperature. Specifically, the inclusion of 200 mg/kg COS led to a decrease in Malondialdehyde content, which suggests reduced oxidative stress, increased activities of antioxidant enzymes Superoxide Dismutase and Glutathione Peroxidase, and increased level of the anti-inflammatory cytokine Interleukin-10 in vital organs. The 100 mg/kg COS also showed improvements, but these were more significant at the 200 mg/kg level.	(Lan et al., 2020)
0, 50, and 100 ppm of COS	Growth performance, blood parameters, carcass traits, fatty acid composition of breast meat, and apparent nutrient digestibility in broiler chicken	Diets added with 50 ppm COS showed a higher average live weight gain, decreased feed intake, and improved carcass yield (higher dressing percentage and increased breast and leg weight) compared to the control group. They also experienced lower total cholesterol, low-density lipoproteins, very low-density lipoproteins, and triglycerides. The 100 ppm COS group also improved but was less marked than the 50 ppm COS group. Therefore, the 50 ppm dietary COS seemed more effective in this study. However, it did not significantly affect key growth performance parameters such as live weight, average weight gain, and feed conversion.	(Tufan and Arslan, 2020)
0, 262, 350, 437 g/ton feed	Growth performance, gut morphology, and serum biochemistry	The serum glucose level was significantly lowered in the 350g/ton and 437g/ton COS groups compared to the control group, which could indicate improved health. The serum concentrations of total protein, albumin, and globulin were gradually increased in all treatment groups, along with the increase in COS dose rates compared to the control group. The 437g/ton COS group had the highest total protein value. The aspartate aminotransferase (AST) liver enzyme level increased with the dose rate, particularly in the 437g/ton COS group.	(Ayman et al., 2022)
0, 400 mg/kg feed	Rectal and surface temperature, oxidative status, the expression of mapk-nrf2-are signaling pathway-related genes, and meat quality	Dietary 400 mg/kg COS and were exposed to acute heat stress (the AHS-C group). Decreased reactive oxygen species and malondialdehyde content compared to the acute heat stress (AHS group). Increased breast muscle pH both at 45 minutes and 24 hours postmortem in comparison to the AHS group. Improved redness of the meat, cooking loss, and shear force compared to the AHS group. Increased catalase activity compared to both the AHS and control groups.	(Chang et al., 2022)

0, 600 mg/kg feed	Hepatic antioxidant capacity, inflammatory response, and lipid metabolism in heat-stressed broilers. In addition, they used body weight, liver weight, abdominal adipose weight, average daily feed intake, average daily gain, and feed conversion ratio as parameters for evaluating the effects of COS on broilers under heat stress conditions	Dietary COS 600 mg/kg feed had beneficial effects on heat-stressed broilers' growth performance and liver health. It improved antioxidant capacity, inhibited the inflammatory response, down-regulated lipogenesis-related genes, and up-regulated lipolysis-related genes, helping alleviate hepatic lipid metabolism disorders induced by heat stress. These results provide a theoretical basis for using COS to treat heat-stress-induced hepatic lipid metabolism disorders in broilers.	(Lan et al., 2023)
0, 1, 2, and 3 g/kg feed	Antioxidative capacity, anti-inflammatory impact, growth performance, and haematological and biochemical indices	Increased levels of COS from 1 g/kg feed to 3 g/kg feed exhibited a decrease in malondialdehyde (MDA) content, thus showing increased antioxidative capacity. Dietary COS 3 g/kg feed showed the lowest levels of pro-inflammatory cytokines (IL-1β and TNF-α) and the highest level of anti-inflammatory cytokines, indicating a more potent anti-inflammatory effect with increasing COS level. Dietary COS 2 and 3 g/kg led to improved body weight gain compared to the COS 1 g/kg feed, indicating that higher doses of COS may improve growth performance more effectively. A decrease in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, and triglycerides was seen across all dietary COS levels. COS 3 g/kg feed showed the most significant decreases, thus reflecting better liver health with increased COS level. Decreased ratios of right ventricle to body weight and right ventricle to total ventricle were seen with increased COS levels, indicating that higher doses of COS may have more substantial cardioprotective effects.	(Fathi et al., 2023)

COS; chitosan oligosaccharides

Figure 1 demonstrates the role of chitosan oligosaccharides (COS) as dietary antioxidants in broiler chicken nutrition. These include a significant decrease in low-density lipoprotein (LDL) and improved immunity and antioxidant capacity. This dietary COS improves development and nutrient digestibility in broiler chickens by reducing inflammation and oxidative stress. The outcomes of the broiler chicken's increased weight gain, decreased feed intake, and increased carcass production are evident. Additionally, it raises serum glucose and protein concentrations in broilers, suggesting that it may have cardioprotective benefits and improve general health.

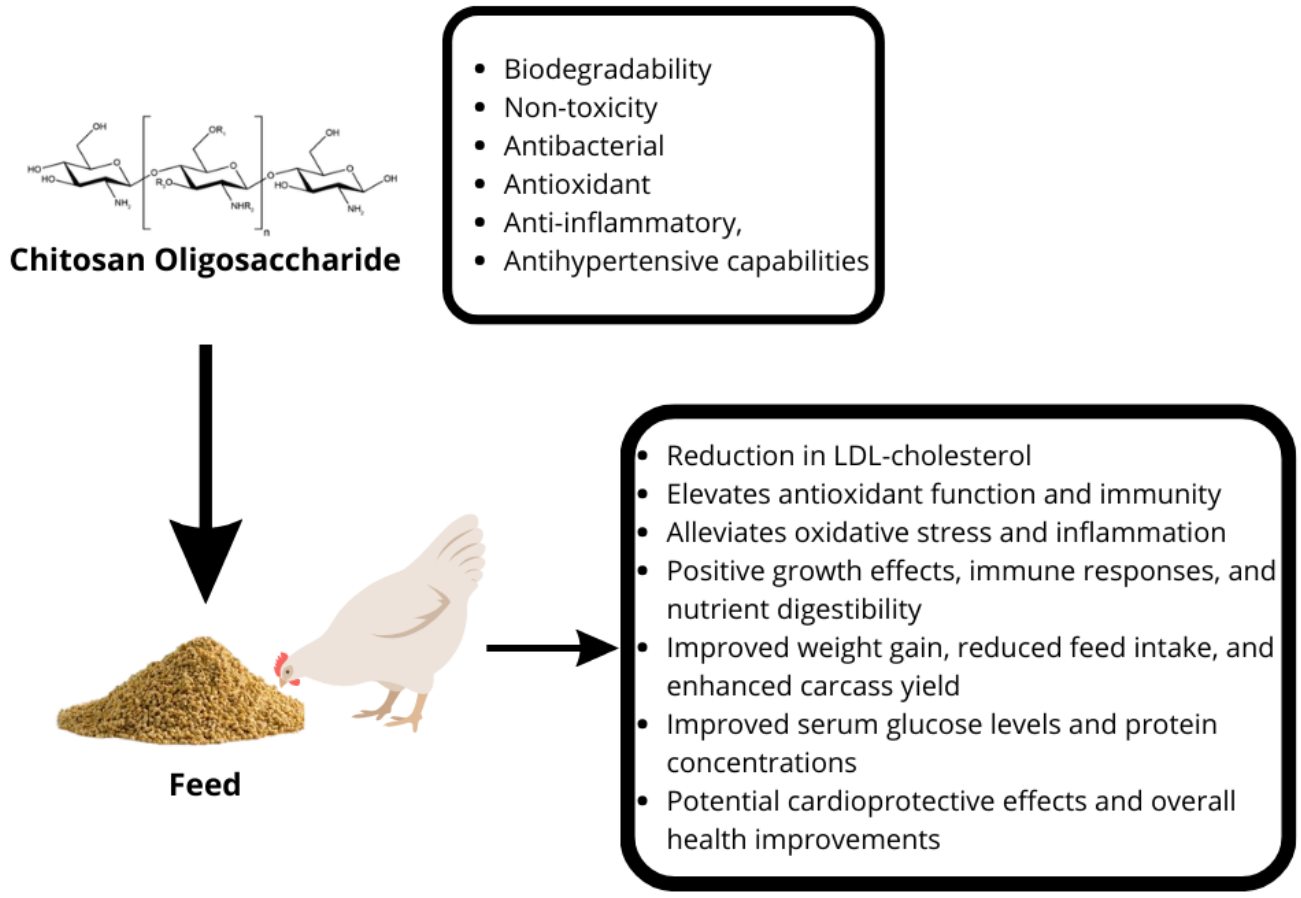

Figure 1 - Role of chitosan oligosaccharides as dietary antioxidants in broiler chicken nutrition

Dietary chitosan oligosaccharides for enhanced broiler performance

The dietary of 125 mg/kg of chitosan oligosaccharides (COS) increased average daily gain (ADG) by 5.9% and improved nutrient digestibility by enhancing gut function (Li et al., 2007). Moreover, the research conducted by Osho and Adeola (2020) presents substantial evidence of the advantageous impacts of dietary COS at a dosage of 1 g/kg feed on broiler chickens, particularly when subjected to stress conditions generated by dexamethasone (DEX). According to a study conducted by Osho and Adeola (2020), including dietary DEX resulted in a significant reduction of 75% in the average body weight gain of broilers that did not receive dietary COS. Nevertheless, upon administering a diet enriched with 1 g/kg COS to the broilers, it was shown that the average body weight gain was reduced by approximately 49%. Consequently, the dietary COS exhibited a statistically significant reduction in the weight loss associated with stress. A similar tendency was also noted in the intake of feed. The broilers exhibited a notable decline in feed intake during periods of DEX-induced stress. However, the extent of this loss in feed intake was comparatively less prominent when the broilers were provided with a dietary COS. Similarly, Tufan and Arslan (2020) reported that dietary COS improved the carcass yield, particularly at the 50 ppm level. Furthermore, according to Fathi et al. (2023), dietary COS 3 g/kg feed showed the best feed conversion ratio.

Osho and Adeola (2020) also reported that dietary chitosan oligosaccharides (COS) effectively alleviated the impact of dexamethasone on the ileal digestibility of dry matter and energy in broiler chickens. It indicates that including COS in the broilers' diet contributed to preserving their capacity to effectively absorb nutrients (dry matter and energy) from their feed, even when exposed to stressful conditions generated by dexamethasone (DEX). The administration of COS was found to positively impact gut health, as indicated by observed alterations in the jejunum's morphology, a small intestine component. Dietary COS resulted in a favourable impact on the morphology of the jejunal region, as seen by the enhancement of villus structure, hence augmenting the capacity for nutrient absorption. Additionally, the COS diet positively affects maintaining a harmonized intestinal barrier function, which may confer protection against the invasion of pathogenic microorganisms in the gut. Similarly, Fathi et al. (2023) reported the positive impact of COS on the intestinal digestibility of dry matter and energy. However, a recent study reported that dietary COS decreased the digestibility of dry matter and crude fat in the dosage 100 ppm COS group compared to the no addition of COS group in broiler. Similarly, organic matter digestibility was reduced in the dosage of COS 50 ppm and 100 ppm groups compared to the no addition of COS group (Tufan and Arslan, 2020).

The dietary chitosan oligosaccharides (COS) has been shown to alleviate the adverse effects of stress on gut health. It improves growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzymes (Osho and Adeola, 2020). COS have also been found to regulate intestinal microflora, enhance protein digestion and absorption, and improve growth and feed conversion efficiency in broiler chickens (Nuengjamnong and Angkanaporn, 2018). Chitosan oligosaccharides exhibit antibacterial properties and can penetrate biofilms, leading to enhanced biofilm killing (Lu et al., 2014). They also perform various biological activities, such as inhibiting the growth of bacteria and fungi, exerting antitumor activity, and acting as immunopotentiation effectors (Choi et al., 2004).

Impact of chitosan oligosaccharides on biochemical and antioxidative parameters in broilers

The potential biological features of chitosan oligosaccharides (COS) have garnered researchers' interest. The evaluation of the antioxidant activity of COS involved the utilization of various indices, including Glutathione Peroxidase, Superoxide Dismutase, Glutathione, Total Antioxidant activity, Inhibition of Hydroxy Radical, and Malondialdehyde. Xiaocong et al. (2017) emphasized the positive impact of dietary COS on the antioxidative capabilities of broilers. This observation was further supported by Osho and Adeola (2020), who reported increased activities of specific enzymatic antioxidants that play a crucial role in safeguarding cells against oxidative stress. Moreover, Deng et al. (2008) underscored the significance of COS in enhancing the immune response of broiler chickens, while Al-Surrayai and Al-Khalaifah (2022) shed light on its diverse range of advantages, encompassing antibacterial characteristics as well as its ability to regulate lipid levels.

Chitosan and its derivatives, such as chitosan oligosaccharides (COS), have been extensively recognized for their diverse biological activities. One of the distinguishing characteristics of chitosan is its capacity to modulate lipid metabolism, potentially through its interactions with lipids and bile acids, so it interferes with lipid digestion and absorption. According to the postulation of Keser et al. (2012), these qualities may be responsible for the observed decreases in low-density lipoprotein (LDL) cholesterol. Additionally, the antioxidative mechanism of COS may arise from its polycationic properties, which allow it to effectively counteract reactive radicals, thus safeguarding cells against oxidative harm.

Dietary chitosan oligosaccharides (COS) have demonstrated favourable results in yellow-feather broilers exposed to elevated ambient temperatures. According to the research findings, the antioxidative properties of COS are mostly attributed to its ability to eliminate reactive oxygen species. COS exhibits a protective effect against lipid degradation by reducing Malondialdehyde levels. Furthermore, the augmentation of antioxidant enzymes such as Superoxide Dismutase and Glutathione Peroxidase suggests that COS plays a role in bolstering the body's intrinsic antioxidative mechanisms (Lan et al., 2020).

Moreover, the anti-inflammatory properties of chitosan oligosaccharides (COS) can be shown in its capacity to regulate cytokines. The observed effect is supported by a reduction in pro-inflammatory markers and an elevation in anti-inflammatory markers, as demonstrated in the study conducted by Lan et al. (2020). Stress, including stress generated by exposure to low temperatures, has the potential to trigger a series of inflammatory reactions within the broiler. In the circumstances mentioned earlier, COS has exhibited noteworthy characteristics. The study conducted by Fathi et al. (2023) demonstrated that dietary COS can mitigate the adverse effects of stress by modulating oxidative stress indicators and promoting lipid profiles.

Dietary chitosan oligosaccharides as a strategy to mitigate stress and inflammatory responses in broilers

Dietary chitosan oligosaccharides (COS) have attracted considerable attention due to their potential involvement in altering physiological responses during periods of stress. The study by Osho and Adeola (2020) aimed to examine COS's impact on broilers' mRNA expression patterns. Their study revealed an increased expression of essential proteins, including zonula occludens-1, zonula occludens-2, claudin-1, and occludin. These proteins play a crucial role in preserving the integrity of the gut epithelium by regulating tight junctions. The observed modification suggests a potential alleviation of gastrointestinal distress. Moreover, the study conducted by the researchers demonstrated a reduction in the levels of pro-inflammatory markers, including tumor necrosis factor-alpha, interferon-gamma, and toll-like receptor-4, in individuals affected by coccidia. This finding suggests that COS possesses anti-inflammatory properties.

The study by Chang et al. (2020) aimed to evaluate the effectiveness of chitosan oligosaccharides (COS) in mitigating the adverse effects of heat stress in broiler chickens. In addition to maintaining growth parameters and liver functions, COS has shown direct antioxidative capabilities. As mentioned above, the phenomenon was observed through an increase in muscle glycogen levels, indicating a decrease in muscle glycogen glycolytic metabolism and an improvement in muscle antioxidant defences. Specifically, the study detected an increase in the levels of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as a decrease in the concentration of malondialdehyde, which is a recognized indicator of lipid peroxidation. Chitosan Oligosaccharides' antioxidative capacity becomes vital due to the heightened oxidative stress under unfavourable settings, resulting from escalated reactive oxygen species (ROS) production. The study by Lan et al. (2020) supports the idea that COS effectively reduces oxidative stress indicators and regulates the inflammatory response in severe stress situations.

In a following investigation by Lan et al. (2023), a more comprehensive analysis was conducted to elucidate the effect of chitosan oligosaccharides (COS) in broiler chickens subjected to heat stress. The focus was on mitigating the adverse effects caused by stress, leading to enhancements in growth patterns and physical measurements. The anti-inflammatory effects of COS were seen by regulating inflammatory markers, resulting in a decrease in IL-1β and IL-6 levels and an increase in IL-10 levels. Furthermore, the compound COS enhanced the antioxidant defence mechanisms, highlighting its potential to alleviate the effects of heat stress.

In the study by Swiatkiewicz et al. (2015), they investigated the potential utilization of chitosan and its oligosaccharide derivatives in the context of chicken and swine feed. The data made by the researchers emphasized the potential of chito-oligosaccharide in augmenting the immune response in hens, surpassing the efficacy of traditional supplements such as chlortetracycline.

Influence of chitosan oligosaccharides on liver function and metabolic health in broilers

Numerous research studies have shed light on the therapeutic effectiveness of chitosan oligosaccharides (COS) in regulating hepatic functions and mitigating oxidative stress. The study conducted by Lan et al. (2023) provided a comprehensive understanding of the effects of dietary COS on the upregulation of nuclear factor erythroid related factor 2 (Nrf2) and catalase (CAT) activity, which play a crucial role in antioxidant activity. The observed increase suggests that COS may have the ability to enhance the liver's antioxidant capacity, protecting it from oxidative stress and its negative consequences. Interestingly, COS also demonstrated proficiency in regulating lipid metabolism. Through the downregulation of genes involved in lipogenesis, such as sterol regulatory element-binding protein 1c (SREBP-1c), acetyl-coenzyme carboxylase (ACC), and fatty acid synthase (FAS), and the simultaneous upregulation of genes associated with lipolysis, COS appears to have the potential to correct disruptions in hepatic lipid metabolism, particularly those caused by heat stress. The decrease in blood triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels demonstrated the regulating ability of COS. These changes are commonly observed under heat stress and indicate a disruption in lipid metabolism.

Consistent with the earlier research, Chang et al. (2020) emphasized the antioxidative characteristics of chitosan oligosaccharides (COS). The research elucidated that COS can enhance the functioning of key antioxidant enzymes, hence strengthening the body's defence mechanism against oxidative stress. Additionally, COS may mitigate excessive fat accumulation in the liver, thereby protecting against developing fatty liver illnesses. The preventive nature of COS was further supported by the observed decrease in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) - enzymes that generally increase in cases of liver diseases.

In their study, Lan et al. (2020) showed the efficacy of chitosan oligosaccharides (COS) as a powerful antioxidant agent, thereby highlighting the significance of further exploration in this area. The study demonstrated a decrease in the

levels of malondialdehyde (MDA), an indicator of reduced oxidative damage and lipid peroxidation in the liver, following the addition of COS. Simultaneously, the administration of COS increased the levels of crucial antioxidant enzymes such as Superoxide Dismutase and Glutathione Peroxidase. These enzymes are crucial in neutralizing harmful oxidative substances and reducing oxidative stress. Although the research did not explicitly investigate the impact of COS on lipid metabolism, the observed decrease in MDA levels suggests a potential reduction in lipid peroxidation, indicating a potentially beneficial influence on lipid metabolic pathways.

Ayman et al. (2022) provided a comprehensive analysis of the various changes in blood biochemical markers following chitosan oligosaccharides (COS) administration. Their findings shed light on the impact of dietary COS on liver function, hepatic antioxidant capacity, and lipid metabolism. The COS was observed to reduce serum total cholesterol and triglyceride levels, potentially inhibiting cholesterol production, facilitating lipid excretion, or modulation of lipid metabolic enzymes. Furthermore, the increased serum concentrations of protein, albumin, and globulin following the administration of dietary COS suggest an improvement in protein metabolism, possibly due to higher protein digestibility. Further inquiry is warranted to explore the ancillary observation of a minor increase in aspartate aminotransferase (AST) levels with a higher dosage of corticosteroids. However, it is worth noting that these levels remained within physiological limits, suggesting that this effect is benign in origin.

Evaluating the implications of chitosan oligosaccharides on meat quality and carcass traits

Recent research has demonstrated that including chitosan oligosaccharides (COS) as a dietary for broiler chickens has significantly impacted muscle quality and carcass characteristics. In a study conducted by Chang et al. (2020), it was noted that broiler chickens experiencing heat stress and receiving dietary COS demonstrated an elevated muscle pH after 24 hours postmortem. This observation is considered advantageous for meat quality, as it is associated with enhanced water-holding capacity, texture, and overall meat quality. The increase in muscle glycogen levels resulting from dietary COS enhances the muscle's capacity for energy storage. In addition, it was noted that heat stress with COS led to a decrease in lactate content, potentially influencing the higher pH values seen and thereby improving the overall quality of the meat. One notable finding from this study is that adding COS can effectively decrease cooking loss in broilers, indicating that the meat preserves a more significant proportion of its weight during the cooking process, resulting in a more succulent final product.

Tufan and Arslan (2020) investigated the impact of chitosan oligosaccharides (COS) on carcass features. The researchers observed notable improvements in the dressing percentage of broilers administered COS at 50 ppm and 100 ppm compared to the control group. Upon analyzing the components of the carcass, it was seen that there was a significant increase in the weight of the wings for both groups exposed to COS concentration. Additionally, there was a notable elevation in the weight of the breast and legs, specifically for the group exposed to a COS concentration of 50 ppm, compared to the control group. Nevertheless, introducing dietary COS did not significantly alter the fatty acid composition of the breast meat. However, the following study should have offered additional information regarding muscle pH, redness, cooking loss, or shear force, which may have resulted in an incomplete comprehension of COS's effects on meat quality.

The findings of Chang et al. (2022) provide additional evidence in support of the results mentioned earlier, indicating that the pH levels measured at 45 minutes and 24 hours after death were comparatively higher in the group dietary COS compared to the group exposed exclusively to heat stress. The association between an increased pH level in meat and enhanced meat quality is commonly observed. Furthermore, the intensity of the red colouration in the meat, a characteristic that consumers commonly associate with the state of freshness, exhibited greater prominence in the group that received dietary COS. The experimental group demonstrated a reduction in cooking loss, suggesting improved moisture retention during cooking, resulting in a more succulent meat product. Furthermore, it was observed that the group addition with COS demonstrated a decreased shear force, indicating a higher level of tenderness in the flesh.

The potential methods by which COS enhances meat quality may be linked to its capacity to enhance antioxidant defences, alleviate oxidative stress, and strengthen immunological function, as proposed by Domínguez et al. (2019). The results, as a whole, emphasize the potential of COS addition in enhancing the quality of poultry meat. However, additional comprehensive investigations may be necessary to understand the full range of its impact.

CONCLUSION

Dietary chitosan oligosaccharides (COS) in the feed of broiler chickens have been found to have several advantages, such as improved growth performance, increased antioxidative ability, and enhanced immunological responses. It can be concluded that dietary COS in broiler chickens in doses ranging from 200 mg to 1000 mg/kg feed has a positive effect on growth performance, antioxidative properties, regulation of lipid metabolism, ability to mitigate stress, impact on meat quality, and carcass traits, but exceeding 2.5 g/kg feed may significantly impair in growth performance in the broiler.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

R. P. Harahap had the idea for the article and drafted it, and performed the literature search.

M. M. Sholikin performed supervision and editing.

Sadarman critically revised the work.

Conflict of interests

The authors declare that they have no competing interests.

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PHYSICOCHEMICAL COMPOSITION OF CRIOLLO AND CRIOLLO X SAANEN GOAT MILK ACCORDING TO AGE AND PARITY IN THE CENTRAL HIGHLANDS OF PERU

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ABSTRACT: Present study aimed to analyze the physicochemical composition of goat milk based on the type of animal, age, and parity in Chupuro, Junin region in the country of Peru. A total of 24 goats were randomly selected, and divided into two groups: 12 native goats and 12 native goats crossed with Saanen. These groups (G1, G2, and G3) included three age categories (1.8, 2.5, and 3.2 years) and three parity levels (first, second, and third parity). The animals were monitored and fed considering their conversion and nutritional requirements. Before the morning milking, 100 ml of milk was extracted in sterile bottles, followed by agitation for 3 to 5 minutes, and the samples were transported using conservation and cooling techniques in a thermal box with ice cubes. These samples were analyzed in the special laboratory. Measurements of pH, acidity, density, lactose, total solids, fat, and protein were conducted using milk analyzer. The findings for the Criollo breed revealed a pH of 6.35 ± 0.31, a lactose concentration of 4.35%, total solids of 11.62 ± 1.31%, protein content of 4.12 ± 0.35%, and fat content of 3.40 ± 0.91%. In comparison, the Criollo x Saanen crossbreed exhibited a pH of 6.43 ± 0.13%, a lactose concentration of 4.45%, total solids of 12.63 ± 0.92%, protein content of 4.26 ± 0.28%, and fat content of 3.95 ± 0.69%. The results indicated that there were no significant differences in the types of milk from native goats and native goats crossed with Saanen. However, significant differences ($P<0.05$) were observed in density, lactose, total solids, fat, and protein between groups of different ages and parity levels. Crossbreeding with the Saanen breed is well received in the region, as it serves to improve milk production, with favorable percentages of fat, protein, lactose, and total solids.

Keywords: Milk quality, Native goats, Parity, Physicochemical composition, Saanen breed.

INTRODUCTION

The caprine species has gained prominence in global markets (Villalobos, 2005; Bidot, 2017). Goat milk constitutes approximately 3% protein, representing a significant protein contribution to the diet, particularly for pregnant or lactating mothers. Additionally, goat's milk serves as a highly digestible food source (Haenlein, 2004; Ocampo et al., 2016). Moreover, goat farming in rural areas of developing countries contributes to the production of meat, milk, skin, fur, and manure (Resendiz et al., 2021).

Ninety-seven percent of the caprine population is concentrated in Asia, Africa, and Latin America, with Asia holding the majority at approximately 51% of the total population (FAOFAST, 2023). The Mediterranean region stands out as the primary hub for dairy goats, with India, Bangladesh, and Sudan emerging as the key producers of goat milk (Rai et al., 2001; FAOFAST, 2023). In Latin America, the largest caprine populations are observed in Brazil, Mexico, Argentina, Peru, and Bolivia, engaging in activities that serve as both economic drivers and essential food sources for human consumption (Miranda, 2021). In contrast to the advanced production technologies employed in Asian countries (Shinde et al., 2016), Latin American caprine production predominantly relies on traditional systems, utilizing native breeds by being a predominant breed and often operating with limited or no technological interventions (Montesinos et al., 2018; Rodríguez and Ortiz, 2020).

In Peru, the estimated caprine population is 1,771,630 heads, with the highest percentage located in the Piura region (19.5%), followed by Ayacucho (11.7%), Huancavelica (9.7%), Ancash (9.6%), and Lima (9.3%) (Management, 2019). The composition of goat milk is influenced by factors such as breed, environment, number of births, and age of the goat, among others (Guo et al., 2001; Dewettinck et al., 2008; Chilliard et al., 2014). The pH of milk is slightly acidic, due to the presence of citric acid, carbonic anhydride, casein, lactalbumin, phosphates, and chlorides (Cabrera-Beltran et al., 2022), contains 13% more calcium (Ocampo et al., 2016), and enhances the absorption of copper and iron (Da Silva et al., 2015).

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It is known that the milk from Creole and Saanen goats is a whitish, thick liquid, whose characteristics vary significantly based on the breed, diet, lactation period, and health status of the mammary gland of the animal (Chilliard et al., 2014) Furthermore, goat milk possesses superior organoleptic characteristics and a notable advantage in absorbing odors, in compared to other species such as cattle (Dewettinck et al., 2008).

The pH of cow's milk is acidic, whereas goat milk is alkaline (Cabrera-Beltran et al., 2022). The protein content is associated with phosphates, serving as a buffer in individuals with gastric ulcers and causing fewer allergic reactions, such as intolerance (Collard and McCormick, 2021). Additionally, goat milk contains 13% more calcium than cow's milk (Ocampo et al., 2016) and enhances the absorption of copper and iron (Da Silva et al., 2015). Conjugated linoleic acid and coenzyme Q are also present in goat milk, conferring anticancer properties. Conjugated linoleic acid has been the subject of studies suggesting that it may have inhibitory effects on the growth of certain types of cancer cells and tumor formation (Bidot, 2017).

Limited research exists on the physicochemical composition of goat milk in the regions of Peru, particularly in Junin, which is crucial information for enhancing the productive capabilities of this species to benefit producers. Consequently, the objective of this study was to analyze the physicochemical composition of Criollo and Criollo x Saanen goat milk according to age and parity in the central highlands of Peru.

MATERIALS AND METHODS

Ethical regulations

The procedures and ethics of this research work were based on the "Code of Ethics for Scientific Research". They were authorized by letter N° 005-GRJ-DRA-AAC-PERÚ-2022. Likewise, was conducted by international and national guidelines for the care and use of research animals.

Area study

The study was conducted in the corral located in the Chupuro district (Figure 1), situated 15 kilometers from the city of Huancayo in the Junin region, Peru. The area is positioned on the left bank of the Mantaro River at an altitude of 3175 meters above sea level, with an average annual rainfall of 650 mm (Senamhi, 2023). Prior to commencing the research, visits to the corral were undertaken to identify goats and characterize them based on age and animal type (Criollo and Criollo x Saanen).

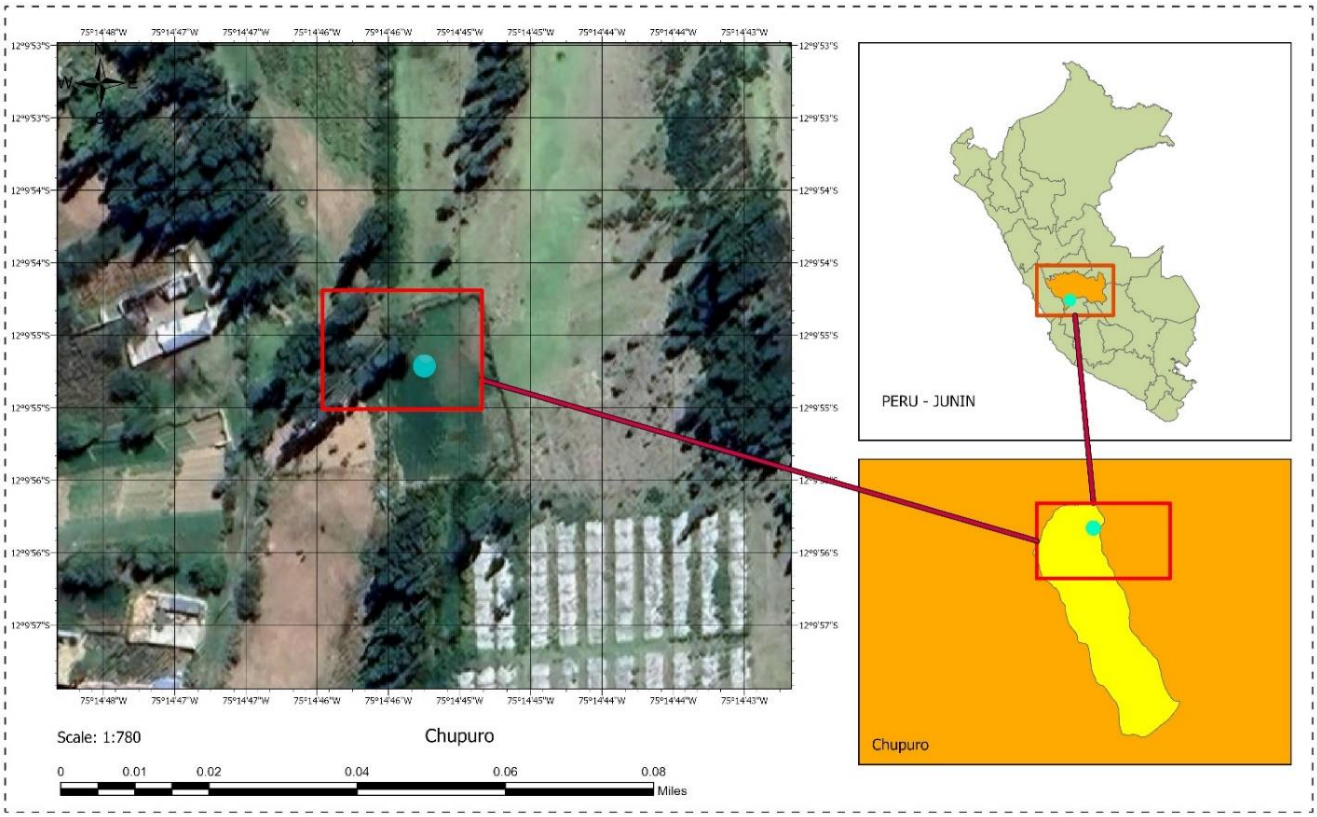

Figure 1 - Location of the study

Animals and distribution

Twenty-four lactating goats were selected, were randomly selected, comprising 12 Criollo and 12 Criollo x Saanen (Figure 2a), categorized into three age groups: G1 (1.8 years and first kidding), G2 (2.5 years and second kidding), and G3 (3.2 years and third kidding); each group consisting of n=8. The animals were individually identified with ear tags for monitoring, and these tags were chosen based on the owner's records to specifically select goats of the specified ages. All animals originated from the same farm and received consistent management practices. They were exclusively pasture-fed with alfalfa (*Medicago sativa*) (Figure 2b). Milking was carried out using a mechanical method, with only one milking per day. The Criollo x Saanen animals belong to the F1 generation resulting from this crossbreeding.

Milk sampling and data collection

A subclinical mastitis test was conducted to eliminate potential confounding factors that could impact the samples. Milk samples were aseptically extracted from each goat before the commencement of milking and post-teat disinfection, utilizing sterile containers. The minimum sample was the total number of animals under investigation with a convenience sample (24 samples). A four-minute agitation of the milk preceded the collection process, adhering to the methodology outlined by Salvador et al. (2016). Subsequently, samples were promptly transferred in a thermal-insulated container with ice packs to ensure proper refrigeration during transit to the Animal Nutrition Laboratory at the Faculty of Zootechnics, National University of the Center of Peru, for subsequent analysis (Guo et al., 2001).

The physicochemical composition of the milk was determined by analyzing fresh milk samples using an automated milk analyzer (Lactoscan S, Milkotronic) (Figure 2d). This apparatus assessed key variables, including pH, density, lactose content, total solids, fat, and protein.

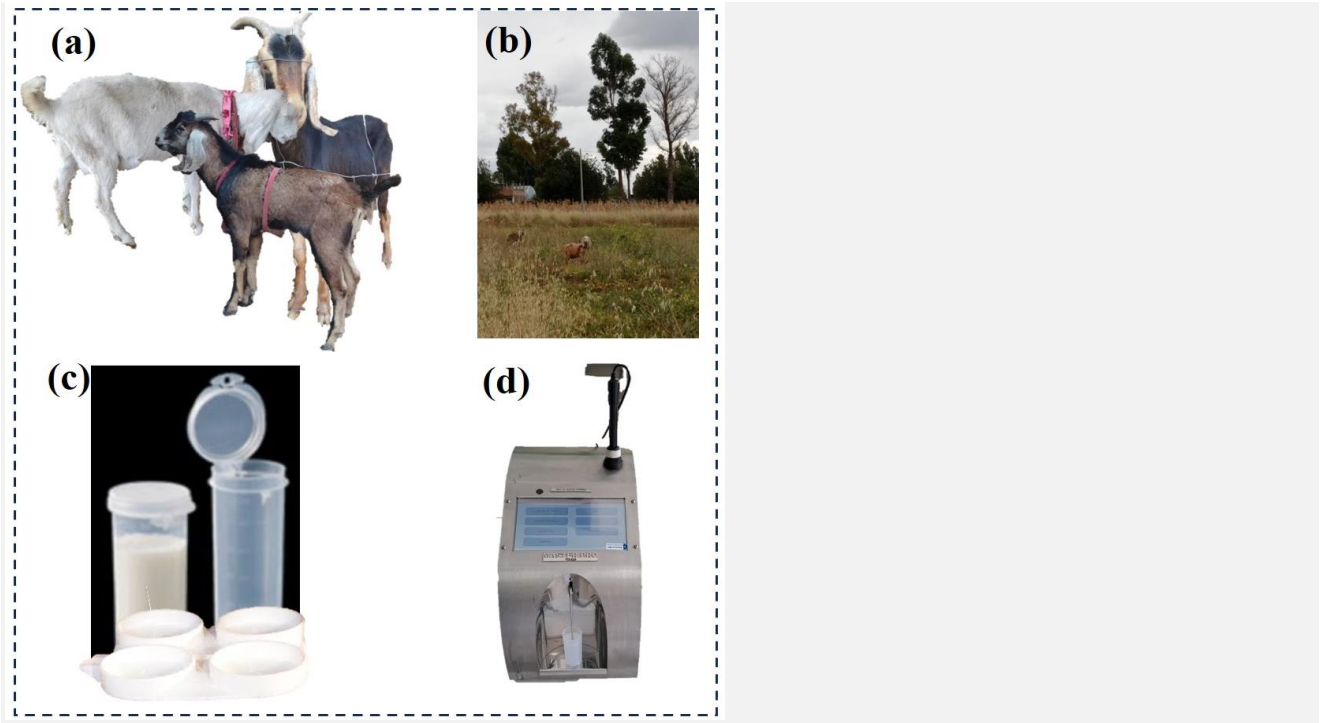

Figure 2 - Procedure: (a) Criollo and Criollo x Saanen goats; (b) Pasture feeding with alfalfa; (c) Samples for analysis; (d) Lactoscan.

Statistical Analysis

The data recorded by the equipment were entered and organized in Microsoft Excel. Differences in ages (1.8, 2.5, and 3.2 years) and parity (first, second, and third) were assessed using analysis of variance (ANOVA), followed by a Tukey post-hoc test. The model used was: $Yij=\mu+\tau i+\beta j+eij$, where Yij is the response variable (physicochemical composition), μ is the overall mean, τi is the effect of ages, βj is the effect of parity, and eij is the experimental error. A significance level of ($p < 0.05$) was considered indicative of a significant difference. Statistical analyses were conducted using the open-source software SPSS 23 (Maswar, 2017).

RESULTS AND DISCUSSION

Physicochemical composition by parturition and age

Table 1 illustrates the physicochemical composition of goat milk, revealing significant differences ($p < 0.05$)

attributed to age and parity. A higher protein percentage is observed in goats aged 3.2 years (third parity), followed by those at 2.5 years (second parity) and 1.8 years (first parity), with values of 4.37%, 4.30%, and 4.25%, respectively. These findings surpass those reported by Alpízar-Solís and Elizondo-Salazar (2019), who documented a protein percentage of 3.27%, likely due to their use of mixed Saanen x Nubian x LaMancha breed goats. Collard and McCormick (2021) also recorded a protein percentage of 3.5%. Similarly, Maldonado-Jaquez et al. (2017) reported 3.37% protein in stall-fed goats, highlighting a decrease in protein concentration in confined goats (Salinas-González et al., 2015). In contrast, these results fall below those reported by Tarazona et al. (2020), who noted a protein content of 5.45%, attributable to the use of Dorper breed sheep. The observed variations in protein content can be attributed to breed and parity differences. It is well-documented that protein percentages increase during the peak of production (3 years and 3 parities) due to udder development (Lozano et al., 2021).

Table 1 - Physicochemical composition of goat's milk in relation to its age ± standard errors ($\bar{X} \pm S\bar{x}$).

Parameters	1.8 years (First birth)	2.5 years (Second delivery)	3.2 years (Third delivery)	P-value
pH	$6.69^{a} \pm 0.45$	$6.73^{b} \pm 0.35$	$6.67^{a} \pm 0.10$	0.006
Acidity (ºD)	16.0 ± 0.2	16.0 ± 0.3	16.3 ± 0.2	0.123
Density g/ml	$1.031^{a} \pm 0.0123$	$1.035^{b} \pm 0.005$	$1.037^{b} \pm 0.031$	0.021
Lactose (%)	$4.39^{a} \pm 0.21$	$4.56^{b} \pm 0.15$	$4.68^{c} \pm 0.22$	0.002
Total solids (%)	$11.97^{a} \pm 1.01$	$12.39^{b} \pm 1.41$	$13.2^{c} \pm 0.9$	0.001
Grease (%)	$3.07^{a} \pm 0.14$	$3.39^{b} \pm 0.11$	$3.99^{c} \pm 0.11$	0.001
Protein (%)	$4.25^{a} \pm 0.43$	$4.30^{b} \pm 0.45$	$4.37^{c} \pm 0.45$	0.004

[a,b,c] Means within a column with different superscripts differ significantly (P<0.05).

Table 2 - Milk production by age and number of births (Criolla and Saanen x Criolla) ± standard errors ($\bar{X} \pm S\bar{x}$).

Age	Production (kg/day)	Lactation duration (days)	Milk production kg/lactation
1.8 (first delivery)	$1.6^{a} \pm 0.1$	$180^{a} \pm 0.1$	$288^{a} \pm 1.2$
2.5 (second calving)	$2.4^{b} \pm 0.2$	$180^{a} \pm 0.1$	$432^{b} \pm 0.1$
3.2 (third parturition)	$3.0^{c} \pm 0.2$	$180^{a} \pm 0.1$	$540^{c} \pm 0.1$
P-value	0.0005	0.125	0.321

[a,b,c] Means within a column with different superscripts differ significantly (P<0.05).

Table 3 - Physicochemical characteristics and milk composition between breeds (Criollo and Criollo x Saanen) ± standard errors ($\bar{X} \pm S\bar{x}$).

Parameters	Criollo	Criollo x Saanen	P-value
pH	$6.35^{a} \pm 0.31$	$6.43^{a} \pm 0.13$	0.123
Acidity (ºD)	15 ± 0.05	15 ± 0.03	0.521
Density g/ml	$1.02^{a} \pm 0.006$	$1.035^{b} \pm 0.0008$	0.010
Lactose (%)	$4.35^{a} \pm 0.08$	$4.45^{b} \pm 0.09$	0.001
Total solids (%)	$11.62^{a} \pm 1.31$	$12.63^{b} \pm 0.92$	0.003
Grease (%)	$3.40^{a} \pm 0.91$	$3.95^{b} \pm 0.69$	0.001
Protein (%)	$4.12^{a} \pm 0.35$	$4.26^{b} \pm 0.28$	0.002

[a,b,c] Means within a column with different superscripts differ significantly (P<0.05).

Table 4 - Milk production by breed (Criollo and Criollo x Saanen)

Parameters	Milk production kg/day	Lactation duration (days)	Milk production kg/lactation
pH	$1.8^{a} \pm 0.2$	$180^{a} \pm 0.01$	$324^{a} \pm 1.23$
Acidity (ºD)	$2.5^{b} \pm 0.4$	$180^{a} \pm 0.01$	$450^{b} \pm 2.23$
P-value	0.0012	0.421	0.003

[a,b] Means within a column with different superscripts differ significantly (P<0.05).

Table 1 highlights that the optimal outcomes were observed in goats aged 3.2 years (third parity) for fat, lactose, and total solids at 3.99%, 4.56%, and 13.2%, respectively. These findings closely align with the results reported by Salinas-González et al. (2015), who documented 4.1%, 4.95%, and 13.14% for fat, lactose, and total solids, respectively, in stall-fed goats. Similar results are also noted by Ocampo et al. (2016), reporting percentages of 4.44%, 4.20%, and 12.59% for fat, lactose, and total solids. The numerical variations in results can be attributed to the study's focus on Criollo goats, which exhibit a lower lactose percentage, advantageous for tolerance in humans (Villalobos, 2005). Additionally, goat milk is known to be more digestible due to differences in the size of fat globules (Calvache García and Navas Panadero, 2012; Nayik et al., 2022). The dispersed nature of these globules facilitates easy metabolism by digestive enzymes, enhancing overall digestion (Kondyli et al., 2012; Eseceli et al., 2021). It is noteworthy that the obtained results fall within the stipulated standards for milk derivatives according to CODEX STAN 243.2003 (FAOFAST, 2023).

Regarding fat content (Table 1), the observed percentage (3.99%) underscores the balance between the detrimental effects of cholesterol and the positive contribution of polyunsaturated omega-3 fatty acids, known for their role in preventing cardiovascular diseases (García et al., 2014). Additionally, the presence of linoleic acid, which possesses anticancer properties, contributes to the health benefits associated with this fat composition (García et al., 2014). Maldonado-Jaquez et al. (2017) demonstrated a fat content of 4.3%, working with grazing goats. Generally, grazing leads to higher fat concentrations compared to goats kept in confinement (Mancilla-Leytón et al., 2013). However, fat content is not solely influenced by grazing; it is also dependent on factors such as age, lactation period, and parity (Vargas, 2019; Caroprese et al., 2016). Fat is a principal component of total solids, and both exhibit similar patterns. The Reyes García et al. (2017) reports a fat content of 3.8% for goats, a result consistent with the findings of this investigation.

Regarding density (Table 1), values of 1.037 g/ml, 1.035 g/ml, and 1.031 g/ml were observed for 3.2 (third parity), 2.5 (second parity), and 1.8 (first parity), respectively. These results align with findings reported by (de Oliveira et al., 2021), who documented a density of 1.00 g/mL. This decrease in density is attributed to the creaming process, as fat is a component of total solids. Similarly, it corresponds with the outcomes reported by Gabas et al. (2012), who identified density values around 1.03 g/mL in goats based on total solids and temperature. The density values exhibit a strong dependence on the total solids content, decreasing as the solvent fraction increases (Gabas et al., 2012). According to N.T.P 202.001-2003 (INDECOPI, 2003), the research results fall within the acceptable range defined by Peruvian standards.

Regarding pH (Table 1), similar values of 6.69, 6.73, and 6.67 were reported for 1.8 years (first parity), 2.5 years (second parity), and 3.2 years (third parity), respectively. These results closely align with those reported by Zain (2013), who indicated a pH of 6.67. The slight variation can be attributed to the presence of CO2, citrate, proteins (including casein and whey proteins), collectively known as non-lactic acidity, in freshly milked milk (Zain, 2013). Acidification due to bacterial activity can lead to a decrease in pH below the normal range of 6.5 – 6.7 (Swadayana et al., 2012). Conversely, higher pH values could be indicative of potential mastitis (Kandeel et al., 2019). The identified pH values fall within the normal range for fresh goat milk (6.5 – 6.8) (Miller and Lu, 2019).

In terms of milk production (Table 2), it is evident that goats aged 3.2 years (third parity) achieved superior results, producing 3.0 liters/day over a lactation duration of 180 days, resulting in a total production of 540 kg/lactation. Arnal et al. (2018) emphasized the close correlation between milk production and lactation duration. The age of the goats and the number of parities per lactation significantly influence production levels (Gráff et al., 2018; Zamuner et al., 2020). Furthermore, as shown in Table 3 Criollo x Saanen crossbred goats (2.5 kg/day) outperformed Criollo goats (1.8 kg/day). Multiple studies have consistently demonstrated that dairy goats (breed-specific) exhibit higher production levels compared to Criollo goats (León et al., 2012).

CONCLUSION

The physicochemical composition of goat milk improves as the goat matures, and by the third parity, it exhibits higher milk quality. The production and physicochemical properties of milk are influenced by various factors, among which age, parity (number of births), and the breed of the animal stand out. Crossbreeding with the Saanen breed has demonstrated a positive impact compared to the Criollo breed, gaining widespread acceptance in the region. These crosses significantly contribute to enhancing milk production, displaying favorable percentages of fat, proteins, lactose, and total solids. These outcomes are comparable to those of specialized goat breeds such as the French Alpine, Anglo, and Blonde.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Edgar Garcia-Olarte: Execution of the research; Jordan Ninahuanca Carhuas: Statistical analysis and editing; Maria Antonieta Flores Guillen: laboratory analysis; Armando Aquino Tacza: animal non-monitoring; Erick Esteban Rojas Ramos: data collection.

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Consent to publish

All authors agree to the publication of this manuscript.

Competing interests

The authors have not declared any competing interest.

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IDENTIFICATION OF SOME GENETIC MARKERS AS PRODUCTIVE AND REPRODUCTIVE TRAITS IN UKRAINIAN DAIRY CATTLE BREEDING

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ABSTRACT: Selection based on DNA markers is a breeding technique grounded in the genomic selection value of animals. The aim of the research is to study the genotypic profile of cows of different breeds in Ukrainian breeding in relation to the genes LEP, CSN3, TG5, BLG, and Pit-1 and to identify the probability of using them as markers for reproductive traits in cows. During the study, the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to analyze the genes and determine their polymorphic characteristics. The obtained data indicated that the same gene variants have varying effects in the studied breeds due to their diverse influences on the genomic background. Specifically, the homozygous state of leptin genes (LEP^{CC}) and pituitary-specific transcription factor ($PIT\text{-}1^{BB}$) was observed to impact the reproductive characteristics of cows in the evaluated breeds. Meanwhile, for the genes casein ($CSN3^{AB}$), thyroglobulin ($TG5^{CT}$), and beta-lactoglobulin (BLG^{AB}), the heterozygous state of alleles was found to influence the key reproductive traits of dairy cattle of Holstein origin. The polymorphism of the genes CSN3, βLG, TG, PIT-1, and LEP indicated the presence of genetic potential for the reproductive function of cows and can be utilized as molecular markers in selective breeding, providing significant progress in improving not only the traits of dairy cattle productivity but also reproductive function. Therefore, in the implementation of selective breeding work, it is advisable to consider genotyping for the genes CSN3, βLG, TG, PIT-1, and LEP as an additional criterion for the selection of animals to enhance both their milk and reproductive characteristics.

Keywords: CSN3, Dairy cattle, Genetic potential, Marker genes, Polymorphism, Reproductive function.

INTRODUCTION

Genomic selection has proven its effectiveness in improving the genetic structure for key selection traits in dairy cattle breeding systems. In milk production, the reproductive capacity of *Bos taurus primigenius* plays a key role in the efficiency of production, determining the profitability and cost-effectiveness of breeding dairy cows (Mello et al., 2020). Genomic selection programs have demonstrated their utility in enhancing the genetic gain of characteristics related to the productivity of cattle; however, progress in reproductive traits has been considerably slower, primarily due to low heritability (Keogh et al., 2021). Moreover, traits associated with reproductive capability typically manifest in the third trimester of an animal's productive life. Thus, traditional breeding methods require more time to assess the reproductive potential of individual animals (De Melo et al., 2017). However, even with these restrictions, the level of genetic variability in dairy cattle is significant enough to enable breeding programs to enhance reproductive efficiency (Berry et al., 2014). In the era of genomic selection, methodologies for studying cows are numerically extensive, combining phenotypes with high-yield genomic SNP marker data. This allows the implementation of selection strategies for new functional traits. Another important advantage of genomic selection is the substantial reduction in generation intervals (Mahmoud et al., 2017).

Berry et al. (2014) highlighted that in both dairy and beef cattle, there is a multitude of reproductive phenotypes, but most reproductive traits in dairy and beef cows exhibit low heritability (ranging from 0.02 to 0.04). Reproductive phenotypes in male animals, such as sperm quality, as a rule are more clearly heritable than reproductive phenotypes in females. However, the low heritability of reproductive traits, especially in females, does not imply that genetic selection cannot alter phenotypic indicators. This is evidenced by the recent decline in reproductive performance in dairy cows, partially linked to aggressive selection for increased milk production. Furthermore, antagonistic genetic correlations between reproductive traits and milk or meat productivity are not universally applicable, meaning that simultaneous genetic selection for both increased productivity (milk and meat) and reproductive capability is indeed possible (Berry et al., 2014). Meanwhile, Raven et al. (2014) argue that genome-wide association studies (GWAS) in most breeds of cattle result in large genomic intervals of significant associations, complicating the identification of causal mutations. This occurs due to extensive linkage disequilibrium within breeds of cattle. Since there is less linkage disequilibrium between breeds, multi-breed GWAS may enhance the accuracy of mapping causal variants (Raven et al., 2014).

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Intra-breed analysis indeed offers the advantage of capturing a higher proportion of variation, but multi-breed GWAS leads to a more precise mapping of QTLs that genuinely differ between breeds (Olson et al., 2012). Therefore, the aim of the research was to leverage SNP associations of the genes LEP, CSN3, TG5, BLG, and Pit-1 with reproductive traits in cows of different breeds in Ukrainian selection. The application of a multi-breed strategy in dairy cattle could be beneficial for refining the location of QTLs and determining the likelihood of using data from these genes as markers for reproduction in *Bos taurus primigenius*.

The application of identified Quantitative Trait Loci (QTL) for the genetic evaluation of animal performance traits, as opposed to traditional breeding programs based solely on phenotype and pedigree information, holds significant potential for enhancing selection accuracy and accelerating genetic improvement in animal productivity (Jiang et al., 2010; Kumar, 2017). The development of Polymerase Chain Reaction (PCR) technologies and the integration of molecular-genetic methods in animal husbandry have facilitated rapid analysis of the correlation between gene allelic variants and performance traits. DNA marker technologies enable the identification of genomic regions (Quantitative Trait Loci or QTL) associated with complex genetically determined traits. The implementation of such molecular-genetic procedures in evaluation significantly enhances selection precision, thereby expediting the genetic enhancement of desirable animal qualities (Kusza et al., 2015; Gritsienko et al., 2022).

Over the past decades, numerous studies have been conducted to identify QTL for dairy performance traits in cattle. However, the investigation of the association between Single Nucleotide Polymorphisms (SNPs) of genes and reproductive traits has revealed various discrepancies among researchers (Oikonomou et al., 2009; Trakovická et al., 2013; Cañizares-Martínez et al., 2021). It is known that leptin genes and leptin receptor genes are considered markers for productivity traits in dairy or beef cattle. Therefore, Trakovická et al. (2013) aimed to investigate associations of polymorphisms in LEP and LEPR genes in cattle breeds, such as Slovak and Pinzgau, with production and reproductive traits. The research results indicated that leptin, in particular, is a candidate gene that primarily influences milk production traits and can be implemented in breeding strategies to enhance the productivity of cattle breeds.

At the same time, Oikonomou et al. (2009) presented opposing data. According to the authors, the DGAT1 alleles and growth hormone receptors, responsible for significant increases in milk production, have a negative impact on reproductive function. Additionally, the leptin allele, associated with a significant increase in milk production, was linked to a slight increase in the frequency of endometritis in cows.

Cañizares-Martínez et al. (2021) assessed allele and genotype frequencies of markers in leptin (LEP), pituitary transcription factor (PIT-1), and luteinizing hormone receptor (LHR) genes, examining their impact on reproductive traits and milk productivity in Holstein cattle. The research results showed that polymorphisms in LEP, PIT-1, and LHR may serve as candidates for marker-assisted selection, with alleles A and G being positively associated. Polymorphisms in LEP and LHR positively influenced interval traits and age at first calving, respectively. These findings indicate that these polymorphisms are candidates for selection using reproductive trait markers. Additionally, a significant trend association with the SNP PIT-1 gene was confirmed by the scientists. This polymorphism is strongly linked to aspects of growth and development, particularly with allele A. The AA genotype tends to increase the number of services per conception (NCS), calving to calving interval (CC), and calving to conception interval (CCI) compared to other genotypes.

Al-Shari et al. (2022) investigated the DNA polymorphism of the FGFBP1, leptin, κ-casein, and αs1-casein genes and their association with reproductive function in dromedary camels. The authors concluded that multiple linear regression analysis (MLR) revealed that SNPs in FGFBP1, κ-casein, and αs1-casein significantly influence the age at first calving (AFC), the number of days to first estrus (CCI), the calving interval (CCI), and the number of services per conception (NSC). The coefficients of determination (R2) also indicated that the variability in phenotypic measurements of the investigated traits may be correlated with the identified SNPs in genes associated with reproduction. Except for the leptin gene, which exhibited a monomorphic pattern and did not confirm its influence on reproductive qualities. Ateya et al. (2023) also pointed out that the identified SNPs in the genes of β-lactoglobulin, κ-casein, and DGAT1 could serve as candidates for marker-assisted selection (MAS) for milk composition, productive, and reproductive traits in Holstein dairy cattle.

Therefore, the use of molecular genetic methods in conjunction with traditional animal breeding approaches is a crucial element in achieving a balanced selection process and optimizing animal breeding programs. Our goal was to study markers that increase milk productivity, its quality indicators, animal health, productive longevity and reproductive function, which will facilitate and accelerate breeding progress, indicating its potential.

MATERIALS AND METHODS

Ethical regulation

The rules for handling animals in the experiment fully complied with European animal legislation protection and comfort on farms (Directive No. 95/58 EU “On the protection of farm animals” of the EU Council on 20.07.1998 as amended by EU Regulation No. 806/203 of 14.04.2003, No. 91/630 EC “Minimum standards for cows protection " dated November 19, 1991 as amended by the EU Regulation). Experimental protocol for blood sampling in cows, approved by the local bioethics commission of the Nikolaev National Agrarian University, Ukraine, on Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals This study is reported in accordance with ARRIVE

guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations. The study was conducted as part of the partial completion of scientific work for the degree of Candidate of Science (PhD) in genetics (agricultural science) at the Department of Biotechnology and Bioengineering of the Nikolaev National Agrarian University, Nikolaev, Ukraine. Before proceeding with sample collection, consent was obtained from the management of the enterprise.

Materials

To carry out the research, research groups were formed from breeding cows of dairy cattle of the direction of productivity. These included Ukrainian Red Dairy (URD, n = 32 head), Ukrainian Black-and-White Dairy (UBWD, n = 32 head), and Ukrainian Red-and-White Dairy (URWD, n = 28 head) breeds from the leading enterprise in the southern region of Ukraine, LLC "Kolos-2011" in Ochakiv district, Mykolaiv Oblast.

Methods

The polymorphism of the CSN3, βLG, TG, PIT-1, and LEP genes was determined using the PCR-RFLP method (Alexander et al., 1988; Pedrosa et al., 2021). According to the recommendations of the manufacturer's methodology of T. Maniatis and N. A. Zinovieva, genomic DNA was extracted from peripheral blood using the standard commercial kit "DNA-Sorb V" produced by "Amplisense" (Russia), (Pedrosa et al., 2021). The concentration of DNA was checked in a 2% agarose gel using electrophoresis. For the polymerase chain reaction (PCR) in this study, a reaction mixture with a volume of 10 µL was used: dH2O – 4.3 µL, 5x PCR buffer (15 mMg-1.0 mL) – 2.0 µL, dNTP mix 10x (2 mM each) – 0.8 µL, two primers (70 ng each) – 0.8 µL, Taq polymerase (1 U/µL) – 0.1 µL, DNA 50-100 ng – 2.0 µL.

Restriction products were separated by electrophoresis in a 2% agarose gel, followed by staining with ethidium bromide solution. Visualization of electropherograms was achieved using a digital camera on a transilluminator under UV light, with subsequent photography. Differentiation of the amplicons was conducted based on the molecular weight marker GeneRulerTM 50 bp DNA Ladder, SM0378 (Fermentas, Lithuania) to determine their sizes (Oztabak et al., 2008). For each gene, the temperature regime and the number of PCR amplification cycles were determined. To analyze the polymorphism of structural loci CSN3, βLG, TG, PIT-1, and LEP, restriction enzymes specific to each locus were used. Typing was performed immediately after the PCR analysis (Grobet et al., 1998).

Statistical analysis

Statistical data processing was carried out using the standard package "Microsoft Excel 2013."

RESULTS AND DISCUSSION

Over the past few decades, a widespread decline in reproductive capacity has been observed in dairy cows, partially attributed to unfavorable genetic correlations with key traits selected for milk productivity. Inadequate reproductive performance and an increase in involuntary culling escalate herd maintenance costs and veterinary sanitary measures, resulting in significant additional expenditures. Therefore, genomic selection for female fertility requires careful examination. One challenge in genomic selection for reproductive traits is the complexity and high polygenicity of reproduction in large ruminants. Genes identified as candidates for such traits may also exert pleiotropic effects on other economically important characteristics (Zhang et al., 2023). Hence, the results of this study could positively contribute to the genetic improvement of the reproductive function of dairy cattle and enhance the understanding of genes with pleiotropic effects for intricately conditioned reproductive traits.

Leptin is a protein actively involved in the growth and metabolism of animals, playing an important role in the regulation of feed consumption, energy metabolism, growth, and reproduction in large ruminants. The leptin gene consists of three exons separated by two introns and is localized on chromosome 4 in cattle (Gritsienko et al., 2022). Analyzing reproductive performance indicators in three groups of cows (Table 1) of different breeds during the I-III lactations and beyond, a certain tendency of correlation between respective traits and polymorphism in the LEP gene was identified. In Ukrainian Red dairy cattle, a positive and significant trend is observed in favor of cows with the CT genotype for the service period duration (88.43 ± 33.18 days) and, consequently, the calving index (4.42 ± 1.66 days). However, for the duration of the intercalving period, the difference favored the homozygous CC genotype. While this difference was statistically insignificant, cows with the CC genotype showed a reduction in the calving interval to 369 days.

In Ukrainian Red Pied dairy cows with the CC genotype, during the first lactation, they exceeded cows with CT and TT genotypes in the service period duration (137 ± 106 days) and calving index (6.85 ± 5.30 days). However, in the second lactation, homozygous animals with the CC genotype exhibited a poorer manifestation of this trait compared to other genotypes. Meanwhile, cows homozygous for the TT genotype demonstrated better reproductive performance. Analyzing indicators of the third lactation revealed a similar trend, with homozygous TT cows having an advantage in reproductive parameters. The interval from calving to first estrus was 44.0 days, the udder recovery period was 54 days, and the intercalving period reached 319 days. Accordingly, the calving index value was 2.2. Characteristics of the higher lactation showed similar trends, but in heterozygous cows with the CT in accordance calving interval was 362 ± 27.5 and 438 ±

68.5 days, respectively. It's worth noting that animals with the C allele in their genotype (CC/CT) had an advantage over homozygous cows with the TT genotype in reproductive traits. Ukrainian Black-and-White dairy cows with a homozygous CC genotype were the best in terms of the LEP gene polymorphism and reproductive performance.

In Ukrainian Black-and-White Dairy cows with the LEP^{CT} genotype, during the first lactation, they outperformed homozygous cows with CC and TT genotypes in the service period duration (102 ± 49.9 days), calving index (5.10 ± 2.46), and calving interval (381 ± 50.6 days). As they aged (second and third lactations), homozygous cows with the *CC* genotype had an advantage in reproductive traits: service period duration (79 ± 19 and 166 ± 60 days); dry period (40 ± 22 and 75 ± 15 days), A certain trend was also noted in the duration of the dry period. Animals of all three breeds with the CT genotype had a shorter udder recovery period than homozygous cows with CC/TT genotypes: Ukrainian Red Dairy cows with the CT genotype by 11 days and TT by 6 days; Ukrainian Red Pied Dairy cows with the CT genotype by 25 days and TT by 9 days; Ukrainian Black-and-White Dairy cows by 19 and 18 days, respectively. It is noteworthy that throughout the entire research period, no statistically significant differences were observed in the polymorphism of the LEP gene concerning reproductive quality traits.

Similar data were obtained by Rambachan et al. (2017) It is now well established that leptin may be a strong candidate gene for cost-effective production as it controls traits such as subcutaneous fat thickness, feed intake and reproductive function. It is now well established that leptin may be a strong candidate gene for cost-effective production, as it controls traits such as subcutaneous fat thickness, feed intake, and reproductive function. it is reported that the LEP gene is located on the 4th chromosome in Bosprimigeniustaurus, is synthesized by adipose tissue and is involved in the regulation of feed intake, energy balance, and is responsible for fertility and immune functions of cattle. According to the authors, this polymorphism can be additionally evaluated for selection with the help of markers, and the developed PCR methodology will accelerate the screening of a large number of animals, and improve the selection of animals for reproductive qualities (Javanmard et al., 2008).

Similar data were obtained by Rambachan et al. (2017) which indicated that the SNP LEP/BsaAI significantly affected the gestation period and dry period of the evaluated population of Hariana cows. Therefore, the authors concluded that leptin is a candidate gene that affects reproductive traits and can be used in breeding, a strategy to improve the reproductive function of Harian cattle (Rambachan et al., 2017). One of the main milk proteins is casein, and of particular interest among the four casein milk proteins is β-casein, which contains 209 amino acid residues in the protein chain, and its gene (CSN3) belongs to a cluster of four casein genes located on chromosome 6, and has a decisive role in the quality of milk and its syrupability (Zhang et al., 2023).

During the analysis of signs of the reproductive capacity of cows of Ukrainian selection according to the kappa-casein locus (Table 2), it was established that representatives of the Ukrainian red dairy breed with the homozygous AA genotype differed in better reproductive capacity in terms of lactations. With the exception of the first lactation, where heterozygous AB individuals had a significant advantage, which had the shortest service period (96±33.5 days) and interval between calvings (381±36.7 days), which in turn improved the insemination index (4.84±1.68). During the II-III and higher lactations, the opposite tendency was noticed – the carriers of the homozygous genotype AA were better in all breeding traits, as their clear advantage was observed. Carriers of another homozygous BB genotype were not found in this group of cows during the study.

Individuals of the Ukrainian red-spotted dairy breed, which had the BB genotype, differed in better reproductive capacity at the age of the first and higher lactations, their index of the duration of the service period was 46±0 and 64±0 days, respectively. This affected the values of the insemination index (2.30±0 and 3.20±0), respectively, and contributed to an excellent interval between calvings (329±0 and 342±0 days), respectively. During the second II-III lactations, carriers of the heterozygous AB genotype had an advantage in terms of reproductive ability. Thus, the interval from calving to fertile insemination was 94±20.5 and 95±50.2 days, respectively, the dry period was 54±10.7 and 77±36.0 days, respectively, and the interval between calvings was at the level of 389±34.7 and 385±45.8 days, respectively. This was also reflected in the average values of the insemination index (4.72±1.02 and 4.79±2.51), respectively. In general, it should be noted that carriers of the B allele (AB/BB) have a better reproductive capacity among UCherRM cows, compared to females of the same age with the homozygous AA genotype. Research has not revealed carriers of the homozygous BB genotype among cows of the Ukrainian black-spotted dairy breed. At the same time, cattle of the Ukrainian black-spotted dairy breed, which had the AB genotype, with the exception of the first lactation, had better indicators of reproductive capacity. The duration of the service period in them during the II and III lactations was lower, compared to homozygous $CSN3^{AA}$ cows, by 27 days and 115 days, respectively, and was 111±22.5 and 120±60.7 days, and in the latter case by the second level of probability. The indicator of the insemination index was 5.58±1.13 and 6.00±3.03, respectively, which was a difference of 1.3 and 4.7

According to the data of higher lactation, animals with genotype AB again had significantly better indicators of reproduction than animals with genotype AA. Since no carriers of the homozygous BB genotype were found among the representatives of the UCM, it should be noted that the B allele has a greater influence on the indicators of reproductive capacity for the CSN3 gene, since cows with the AB genotype had better reproductive function values than their homozygous counterparts for the A allele. Similar studies were conducted by Ardicli et al. (2019).

Table 1 - Relationship of LEP Gene SNPs gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	CC			CT			TT		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	124±45.3	137±106	108±10	124±47.9	261±104.6	102±49.9	115±33	193±0	161±76.9
Insemination index, times	6.23±2.26	6.85±5.3	5.40±0.5	6.21±2.39	13.09±5.23	5.10±2.46	5.75±1.65	9.65±0	8.08±3.84
Inter-calving period duration, days	408±48.1	414 ±104.4	369±9.5	406±48.1	441±57.5	381±50.6	399±34.5	485±0	433±69.8
2nd lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	254±157.9	204±114.7	79±19	134±57.1	125±55.8	89±20.5	178±28.5	86±0	179±53.2
Dry period duration, days	62±6.1	45±8.2	40±22	74±28	57±7.9	51±20.1	46±6.5	77±0	53±10.3
Insemination Index, times	12.71±7.89	10.24±5.73	3.95±0.95	6.74±2.86	6.28±2.79	4.48±1.03	8.93±1.43	4.3±0	8.99±2.66
Inter-calving Period Duration, days	445±54.6	418±51.7	362±27.5	398±55.6	378±18.7	377±28.2	466±30	362±0	475±56.7
3rd lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	100±42.2	113±21.8	166±60	111±40	88±15.8	188±130.7	111±53	44±0	181±67.6
Dry period duration, days	77±52.1	70±30	75±15	66±18.9	45±4	51±8.5	73±7	54±0	69±10.4
Insemination index, times	5.03±2.11	5.70±1.28	8.30±3	5.55±2	4.42±0.79	9.43±6.53	5.55±2.65	2.20±0	9.05±3.38
Inter-calving period duration, days	345±10.2	394±26.6	438±68.5	454±70.2	342±10.5	498±105.4	396±50.5	319±0	415±76.7
Higher Lactation	n =4	n =10	n =2	n =7	n =4	n =7	n =2	n =1	n =6
Duration of service period, days	96±59.3	127±49.4	138±88	88±33.2	121±58	219±111.7	129±18	183±0	192±55
Dry period duration, days	45±9.6	69±21.9	73±17	57±15.5	54±9.23	51±5.7	61±3.0	219±0	61±12.4
Insemination index, times	4.83±2.96	6.36±2.47	6.90±4.4	4.42±1.66	6.05±2.9	10.99±5.58	6.45±0.9	9.15±0	9.61±2.75
Inter-calving period duration, days	336±113.5	394±43.5	507±20	430±73.8	353±3.4	498±165.9	415±12	–	431±83.3

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. Leptin (LEP); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

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Table 2 - Relationship of SNPs of the CSN3 gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	AA			AB			BB		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	136±42.5	226±147.6	104 ±32.8	96±33.5	133±37.8	160±89.7	-	46±0	-
Insemination index, times	6.82±2.13	11.31±7.38	5.21±1.64	4.84±1.68	6.68±1.89	8.03±4.48	-	2.30±0	-
Inter-calving period duration, days	419±43.6	453±117.2	377 ±34.5	381±36.7	415±41.5	435±82.17	-	329±0	-
2nd lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	142±49.6	198±113.5	138±71.2	217±123.2	94±20.5	111±22.5	-	167±0	-
Dry period duration, days	66±21.6	51±7.4	48±8.9	65±11.8	54±10.7	54±10.3	-	51±0	-
Insemination index, times	7.11±2.48	9.90±5.68	6.91±3.56	10.86±6.16	4.72±1.02	5.58±1.13	-	8.35±0	-
Inter-calving period duration, days	418±56.8	409±49.8	435±70.5	427±76.2	389±34.7	387±25.0	-	443±0	-
3rd lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	117±41.9	111±23.5	235±85.4	134±61.2	95±50.2	120±60.7 b **	-	109±0	-
Dry period duration, days	73±28.3	51±12.5	62±11.5	134±61.2	77±36	61±12.7	-	46±0	-
Insemination index, times	5.86±2.09	5.57±1.4	11.77±1.27	6.71±3.06	4.79±2.51	6.00±1.03 b ***	-	5.45±0	-
Inter-calving period duration, days	406±57.8	385±42.7	473±116.2	450±50.5	385±45.8	443±69.9	-	385±0	-
Higher Lactation	n =10	n =8	n =9	n =6	n =6	n =6	n =0	n =1	n =0
Duration of service period, days	112±56.1	145±57.7	220±87.6	149±80.8	119±53.7	157±81.7	-	64±0	-
Dry period duration, days	54±10.1	55±7.9	58±11.1	56±15.4	107±44.8	60±11.3	-	109±0	-
Insemination index, times	5.63±2.81	7.26±2.88	11.04±4.38	7.49±4.04	5.95±2.68	7.88±4.08	-	3.20±0	-
Inter-calving period duration, days	400±81.3	411±49.2	471±117.6	402±88	367±23	481±149.5	-	342±0	-

Significant: *=P<0.05; **=P<0.01 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. CSN3 - kappa-casein , Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

The results of their experiments showed that CSN3 affects the length of the independence period and the interval between calvings. The authors also indicate that the SNP of the CSN3 gene was significantly associated with the duration of pregnancy, in addition, the effect of CSN3 on the age of first calving was observed. At the same time, ambiguous results of research by Tsiaras et al. (2005). Thus, the authors did not find any associations between polymorphisms in the CSN3 locus and reproductive capacity. However, there was a tendency to increase the age of cows at the first and second calving with the AB genotype.

The TG5 gene is one of the longest mammalian genes. In cattle it is located in the centromeric region of the 14th chromosome and consists of 37 exons. The TG5 gene has two allelic variants, $TG5^{T}$ and $TG5^{C}$, and three genotypes: $TG5^{CC}$, $TG5^{CT}$, and $TG5^{TT}$. Glycoprotein of thyroglobulin is a precursor of iodothyronine hormones of the thyroid gland, which regulate many physiological and biochemical processes in almost all tissues of the body by regulating gene expression, and affect the performance indicators of cows (Safina, 2018).

Analyzing the relationship between the polymorphism of the TG5 gene and the indicators of the reproductive capacity of cows of different breeds according to the data of I-III and higher lactation (Table 3).

It was established that in all three experimental breed groups there are no individuals with the homozygous TT genotype, which is probably related to the individual characteristics of the animals, and a small frequency of occurrence of this genotype. But at the same time, comparing the effect of the TG5 gene on the reproductive characteristics of cows in the red dairy breed, it should be noted that there is a clear influence of the T allele on the characteristics of reproductive capacity - carriers of the heterozygous CT genotype had better indicators of reproductive function, with the exception of the first lactation, where the advantage was on sides of homozygous CC individuals. At the same time, the advantage of heterozygous organisms in the 3rd lactation according to the main signs of reproduction was probable ($P<0.01$).

Characterizing the influence of the TG5 gene polymorphism on the reproduction characteristics of Ukrainian red-spotted dairy cattle in the section I-III and higher lactations, no dependence was established between the above-mentioned parameters. Since the signs of reproduction had an oscillatory nature of their manifestation, both in terms of lactation and in terms of the investigated genotypes (CC or CT).

That is, the effect of the homo- or heterozygous state of the TG5 gene on the manifestation of indicators of the signs of the reproductive function of Ukrainian red-spotted dairy cows has not been established. The assessment of reproduction indicators and the effect on them of the TG5 gene polymorphism in representatives of Ukrainian black-spotted dairy cattle established the opposite trend - a clear effect of heterozygosity of individuals for the TG5 gene on reproductive characteristics was observed. So, for the I-III lactations by the length of the service period (101, 101 and 50 days, respectively), the dry period (52 and 38 days, respectively), the interval between calvings (377, 331 and 384 days, respectively), as well as the index insemination (5.05; 5.05 and 2.50, respectively) had a clear advantage of heterozygous individuals with the ST genotype, although only one animal fell into this group.

In the scientific literature, there is very little data confirming or refuting the influence of SNPs of the TG5 gene on the characteristics of animal reproduction. According to Deb et al. (2021), relatively similar research results were obtained. Thus, the analysis of the growth dynamics of first-born cows with different genotypes according to TG5 showed that individuals with the TT genotype had an advantage in live weight, and as a result, were fertilized at an earlier age. However, in terms of reproductive qualities, first-born cows with the $TG5^{TC}$ genotype were inferior to cows with other genotypes. After the first calving, heterozygous $TG5^{TC}$ individuals had a shorter service period and a high Doha index. In this follows a similar tendency to our researches.

Beta-lactoglobulin (BLG) is the main protein of cow's milk and affects milk yield, fat and protein content, as well as syrup properties. Until now, several variants of BLG have been identified, but the most common are A and B. The genes for the whey protein are located on two different chromosomes: the BLG gene encoding β-LG on chromosome 11, and the BLG gene encoding α-LA on chromosome 5 (Pedrosa et al., 2021).

Our studies of the indicators of the reproductive capacity of cows of three experimental breeds of Ukrainian selection according to the BLG locus (Table 4) established that in the individuals of the Ukrainian red dairy breed, which have the BB genotype, the indicators of the duration of the service period for the first lactation were significantly better, compared to the carrier females of the same age genotypes AA and AB on 51 days and 73 days, and, accordingly, the indicator of the insemination index was lower by 2.55 and 3.67, and the difference between the indicator of the intercalving period was smaller by 58 and 75 days, respectively. The results of research show a probable effect only during the first lactation. During the second lactation, the difference in the duration of the service period of the above-mentioned genotypes was 108 days and 37 days, but it was unlikely. In general, a better manifestation of signs of reproduction is observed in carriers of the B allele, compared to homozygous AA organisms. Which may indicate a certain influence of beta-lactoglobulin β-LG on indicators of reproductive capacity of cows.

Among Ukrainian red-spotted dairy cows, a similar tendency was found when comparing reproductive traits with the BLG gene polymorphism. Thus, cows that are carriers of the B allele genotype and especially heterozygous organisms - AB, had better indicators of reproductive capacity compared to their homozygous AA counterparts. Thus, their interval before fertile insemination, the dry period and the period between calvings were significantly shortened during the I-III lactations. With the exception of higher lactation, where cows carrying the AA genotype had better reproductive capacity. Accordingly, heterozygous females had a significantly better insemination index, compared to other experimental groups.

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Table 3 - Relationship of SNPs of the *TG5* gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	CC			CT			TT		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	121±38	178±117.53	128±61.6	123±60.9	167±25.5	101±0	-	-	-
Insemination index, times	6.05±1.9	8.93±5.88	6.43±3.08	6.18±3.04	8.38±1.28	5.05±0	-	-	-
Inter-calving period duration, days	404±38.6	426.23±90.2	402±59.5	408±64.4	454±30.5	377±0	-	-	-
2nd lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	181±82.5	166±95.6	128±50.7	123±60.4	114±28	101±0	-	-	-
Dry period duration, days	67±19.4	50±6.8	50±10.1	58±16.2	68±8.5	52±0	-	-	-
Insemination index, times	9.06±1.12	8.31±4.78	6.43±2.53	6.17±1.02 a *	5.70±1.4	5.05±0	-		-
Inter-calving period duration, days	428±60	401±42.1	419±50.9	394±70	419±57	331±0	-	-	-
3rd lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	134±27.5	100±26.1	193±92.5	64±11.0 a *	125±81.5	50±0	-	-	-
Dry period duration, days	63±14.4	62±23.3	63±11.4	99±15.3 a *	50±3.5	38±0	-	-	-
Insemination index, times	6.73±1.38	4,97±1.4	9.66±4.63	3.20±0.55 a **	6.28±4.08	2.50±0	-	-	-
Inter-calving period duration, days	430±27.3	392±40.8	458±100.8	391±17.5 a *	356±37.5	384±0	-	-	-
Higher lactation	n =13	n =13	n =14	n =3	n =2	n =1	n =0	n =0	n =0
Duration of service period, days	130±67.7	120±52.8	197±93	109±70.2	192±9	202±0	-	-	-
Dry period duration, days	57±11.2	63±16.3	59±11.6	38±8	160±59	55±0	-	-	-
Insemination index, times	6.52±3.39	6.0±2.64	9.90±4.65	5.48±3.51	9.60±0.45	10.1±0	-	-	-
Inter-calving period duration, days	395±92.9	386±39.7	454±115.4	427±51.6	–	680±0	-	-	-

Significant: *=P<0.05; **=P<0.01 (compared to the animals of the first control group); a = P<0.05; b= P<0.01 (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly (p<0.05). TG5 thyroglobulin gene (TG-5); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

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Table 4 - Connection of *BLG* gene SNPs with reproductive characteristics of cows

Genotype g.0000 C>T ($X \pm S_x$) / Trait/Indicator	AA			AB			BB		
	URD	URSD	UBWD	URD	URSD	UBWD	URD	URSD	UBWD
1st lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	127±38.1	258±212.5	118±0.0	149±32.9	94±0.0	120±68.8	76±22.3 a **	173±93.4	132±53.3
Insemination index, times	6.36±1.91	12.93±10.63	5.9±0.0	7.48±1.64	4.7±0.0	6.03±0.0	3.81±1.11 a *	8.65±4.67	6.62±2.82
Inter-calving period duration, days	414±39.9	344±15.0	360±0.0	431±33.4	371±0.0	395±58.6	356±21.8	451±94.2	409±58.2
2nd lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	219±104.2	119±48.0	60±0.0	148±54.1	81±0.0	104±26.1	111±51.8	176±106.6	149±63
Dry period duration, days	65±12.7	52±1.0	18±0.0	54±13.0	26±0.0	48±10.5	81±38.4	54±8.5	56±5.2
Insemination index, times	10.98±5.21	5.95±2.40	3.0±0.0	7.43±2.70	4.05±0.0	5.22±1.30	5.56±2.59	8.81±5.33	7.45±3.15
Inter-calving period duration, days	434±66.4	385±0.0	335±0.0	426±57.9	364±0.0	385±22.0	395±53.0	398±43.6	444±62.9
3rd lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	115±41.8	138±0.0	226±0.0	108±39.6	86±28.9	161±127.2	156±51.7	110±1.5	191±69.4
Dry period duration, days	86±27.5	77±0.0	90±0.0	53±19.9	64±24.7	62±13.9	61±15.8	46±0.50	57±8.3
Insemination index, times	5.76±2.09	6.90±0.0	11.30±0.0	5.40±1.98	4.14±1.41	8.05±6.36	7.83±2.59	5.53±0.07	9.55±3.47
Inter-calving period duration, days	414±59.4	418±0.0	507±0.0	415±64.4	379±50.5	472±139.0	450±37.3	385±0.0	447±78
Higher lactation	n =7	n =3	n =1	n =5	n =1	n =6	n =4	n =11	n =8
Duration of service period, days	121±75.3	88±24.0	226±0.0	132±55.1	181±0.0	165±133.4	128±71.0	126±57.3	215±55.4
Dry period duration, days	59±11.9	78±31.00	90±0.0	54±12.1	62±0.0	60±13.8	49±11.8	76±34.6	54±4.1
Insemination index, times	6.06±3.76	4.4±1.20	11.3±0.0	6.63±2.76	9.05±0.0	8.26±6.67	6.40±3.55	6.30±2.86	10.77±2.77
Inter-calving period duration, days	344±107.4	342±0.0	507±0.0	455±76.1	380±0.0	438±155.8	432±46.3	392±44.8	493±110.2

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01. (compared to the animals of the third experiment group with analogues of the second experimental group) Mean values with different superscripts in the column differ significantly (p<0.05). *BLG* - betalactoglobulin; Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD)

Table 5 - Relationship of SNPs of the *Pit-1* gene with reproductive traits of cows

Genotype g.0000 C>T ($X \pm S_x$)	AA			AB			BB		
Trait/Indicator	**URD**	**URSD**	**UBWD**	**URD**	**URSD**	**UBWD**	**URD**	**URSD**	**UBWD**
1st lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	112±42.2	206±177.5	101±0	-	186±91.1	151±80.4	136±39	114±62	86±22.6
Insemination index, times	5.64±2.11	10.30±8.88	5.05±0	-	9.32±4.56	7.58±4.02	6.80±1.95	5.70±3.10	4.34±1.13
Inter-calving period duration, days	396±52.3	481±175.1	379±0	-	417±57.6	426±76.1	420±37.8	394±64.2	357±17.3
2nd lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	180±102.5	247±158	286±0	-	120±46.7	129±38	153±48.3	130±47.6	81±17.8
Dry period duration, days	67±21.2	43±8.6	67±0	-	55±0	52±6.7	64±12.8	58±2	44±13.3
Insemination index, times	9.02±5.12	12.38±7.9	14.30±0	-	6.01±2.33	6.45±1.90	7.68±2.42	6.52±2.38	4.09±0.89
Inter-calving period duration, days	423±66.1	410±55.5	570±0	-	385.7±23.3	421±36.6	420±60.7	434±53.1	356±23
3rd lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	118±54.5	102±28.9	285±0	-	88±22.3	202±100.6	132±40.5	140±54	115±55.1
Dry period duration, days	78±23.8	52.0±12.5	67±0	-	67±33.9	60±10.9	56±18.8	60±8.9	63±14.5
Insemination index, times	5.92±2.72	4.93±1.98	14.25±0	-	4.40±1.12	10.13±5.03	6.63±2.03	7.00±2.7	5.79±2.76
Inter-calving period duration, days	410±44.2	378±40.0	569±0	-	356±26.6	448±118.7	444±82.2	438±29.8	454±42.4
Higher lactation	n =10	n =4	n =1	n =0	n =8	n =9	n =6	n =3	n =5
Duration of service period, days	114±54.1	150±65.5	285±0	-	117±50.1	222±97	147±84.3	133±49.8	142±74.3
Dry period duration, days	53±12.5	52±4.8	67±0	-	93±44.3	56±9.9	57±12.8	74±18	61±13.9
Insemination index, times	5.71±2.7	7.50±3.28	14,25±0	-	5.89±2.5	11.11±4.85	7.35±12.83	6.68±2.49	7.12±3.72
Inter-calving period duration, days	410±45.3	404±58.5	569±0	-	367±22.8	467±125.9	386±153.5	389±34.5	462±131.3

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to the animals of the first control group); a = P<0.05; b= P<0.01 (compared to the animals of the third experiment group with analogues of the second experimental group). Mean values with different superscripts in the column differ significantly. Pituitary-specific transcription factor (Pit-1); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD).

However, in cows of the Ukrainian black-spotted dairy breed, which have the homozygous AA genotype, the indicator of the duration of the service period and the interval between calvings during the II lactation was lower, compared to other homozygous BB organisms - by 89 days and 109 days, respectively, the indicator of the insemination index - at 4.45. A similar trend was observed in the first lactation, although comparatively, with a small advantage. At the time when during the 3rd and higher lactations, better reproductive capacity was characteristic of cows with the heterozygous AB genotype. What can indicate the influence of the state of heterozygosity of the organism for the BLG gene on indicators of the reproductive capacity of cows, which was observed in cows regardless of their breed affiliation.

The association of milk protein genes with the fertility of Holstein cattle was also studied by Peñagaricano and Khatib (2012), who, after correcting for polymorphisms in LALBA and BLG genes, established a significant relationship with successful fertilization and the number of blastocysts, which may indicate a relationship between whey protein genes and cow fertility. At the same time Demeter et al. (2010) confirm that the selection of cows based on the polymorphism of milk protein variants with improved production properties does not have a negative effect on the reproductive performance of cows, but on the contrary, is a promising direction of breeding programs. Because this will allow establishing the most successful combinations of genes to improve reproductive traits.

The Pit-1 or POU1F1 gene is a key transcription factor located on the first chromosome in cattle, weighs approximately 33 kDa, has 5 introns and 6 exons, and consists of 291 amino acids. Pit-1 plays a role in pituitary development and somatic cell proliferation, as well as the secretion of the hormones growth hormone (GH) and prolactin (PRL) in mammals. Since it is related to pituitary hormones, it affects the ovulation rate in cattle (Fındık et al., 2022).

The analysis of the reproductive function of cows revealed the connection of its features with polymorphism in the gene of the pituitary-specific transcription factor PIT-1 (Table 5): a positive trend was observed in favor of cows of the BB genotype according to the insemination index for the 1st and 2nd lactations among the three experimental breeds, as well by the duration of the service and inter-hotel periods, we established a difference in favor of the BB genotype, although it was statistically unreliable.

Cows of the PIT-1^{BB} genotype of three breeds had a shorter service period for I-III and higher lactation, compared to peers of the AA and AB genotypes. Accordingly, this affected the duration of the interval between calvings. And as a result, the insemination index among cows of the Ukrainian black and spotted dairy breed was the lowest, precisely among individuals of the homozygous PIT-1^{BB} genotype. A similar influence of the B allele on reproductive performance indicators was also established among cows of the Ukrainian red-spotted dairy breed - carriers of the B allele genotypes, as well as AB heterozygotes and BB homozygotes, were distinguished by better reproductive qualities in the section I-III and higher lactations, compared to homozygotes analogues of AA.

Among individuals of the Ukrainian red dairy breed, a clear influence of the PIT-1 gene on reproductive qualities has not been established, although cows with the BB genotype have a lower index of insemination in the first and second lactations than animals with the AA genotype, and, accordingly, the interval to fertile insemination and the intercalving period are shorter. The results of studies by Pytlewski et al. (2018) indicated the relationship between the PIT-1 gene and reproductive traits that proved the existence of dependencies between the PIT-1 gene and the reproductive potential and body weight of cows and calves. But according to the authors, more favorable results were observed in homozygous AA calves. Al-Khuzai and Al-Anbari (2018), advise using the PIT-1 gene as a genetic marker to improve reproductive traits of high-fertility sheep. Since a polymorphism was established between this gene and sheep fertility.

CONCLUSION

The identified trends in inter-breed differentiation of polymorphism in the genes CSN3, BLG, TG5, PIT-1, and LEP based on reproductive performance indicators in cows of different Holstein-origin breeds are somewhat diverse and occasionally statistically inconclusive due to the small number of groups of investigated animals carrying a specific genotype, the frequency of which is low in the populations of the studied farm. However, it has been established that the same genetic variants influencing reproductive function have different effects in specific breeds due to the specificity of their genomic background. The influence of the leptin gene on the reproductive traits of cows was noted in carriers of the C allele, who generally exhibited better reproductive function compared to carriers of the A allele. The impact of the homozygous or heterozygous state of the B allele in the CSN3 gene on reproductive indicators was established, as cows of the investigated breeds that were carriers of the B allele (AB/BB) showed better reproductive performance compared to the homozygous genotype AA. The analysis of the polymorphism of the TG5 gene in relation to the reproductive traits of cows of different breeds revealed the absence of individuals with a homozygous TT genotype among the evaluated representatives, which is likely related to individual characteristics of the animals and the low frequency of occurrence of this genotype. However, a clear influence of heterozygosity for the TG5 gene was observed on the reproductive characteristics of most investigated dairy breeds. Research on the reproductive traits of cows from three studied breeds of Ukrainian selection at the BLG locus indicates the influence of the allelic combination of the BLG locus on the reproductive traits of cows, irrespective of their breed. The analysis of the reproductive function in cows revealed a connection between its traits and the polymorphism in the PIT-1 gene. A positive dynamics in reproductive performance was observed in favor of cows with the PIT-1^{BB} genotype for the calving index in the first and second lactation among the three studied breeds. Also a difference in the duration of the service and intercalving periods was noted in favor of animals with the PIT-1^{BB} genotype, although it was statistically insignificant.

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Therefore, the polymorphism of the CSN3, BLG, TG5, PIT-1, and LEP genes can be utilized as molecular markers in selective breeding to ensure expected progress in improving not only the traits related to the milk productivity of cattle but also their reproductive function.

DECLARATIONS

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Authors' contribution

The first three authors should be considered first authors. M. Gill planned, designed, spell checked and led this study. Y. Gritsienko collected data and samples, conducted laboratory tests, analyzed and visualized data. O. Karatieieva described the results obtained, formulated the initial version of the manuscript and critically edited the final version of the manuscript. M. Gil also supervised the data and reviewed the manuscript. All authors read and approved the submitted version of the manuscript.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Competing interests

The authors did not declare any conflict of interest.

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EFFECTIVENESS OF COCONUT MEAT WASTE IN FEED INTAKE, DIGESTION AND PROTEIN RETENTION IN GOATS

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RESEARCH ARTICLE

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ABSTRACT: The objective of the experiment was to determine the proportion of coconut meat waste on feed consumption, nutrient digestibility and nitrogen retention of goats. The experiment was conducted using a Latin square design on 4 male Bach Thao goat (16.2±2.93 kg). Treatments were 4 levels of coconut meat waste (CMW) at 0, 5, 10 and 15% in basal diet of rice distillers' by-product, cabbage waste, *Operculina turpethum* vines, urea and premix. Results indicated that dry matter intake per body weight tended to increase from CMW0 to CMW5 treatment but it was gradually reduced from CMW5 to CMW10 and CMW5 treatments (3.34; 3.50; 3.46 and 3.28, respectively). The ME consumption was higher at supplemented treatments coconut meat waste compared to CMW0 treatment. The nutrient digestibility (%) was gradually increased from CMW0 to CMW15 treatment. Similarly, digestive nutrients tended to increase with increasing coconut meat waste in the diet. The nitrogen retention was numerically lower for the CMW5, CMW10 and CMW15 treatments compared to CMW0, while daily weight gain was not different among treatments. In conclusion, 10% coconut meat waste could be used as an additional source of dietary regimen in goats, without negative effects on animal fattening performance.

Keywords: Agricultural waste, Alternative feedstuff, Digestibility, Local feeds, Small ruminants.

INTRODUCTION

Agricultural by-products and local feeds are very popular in the Mekong Delta from the countryside to market such as cabbage waste, *Operculina turpethum* vines and rice distillers' by-products (Trung and Dong, 2013; Trung et al., 2013; Olson and Morton, 2018). In a previous study, Phong and Thu (2018) found that para grass (*Brachiaria mutica*) can be replaced by cabbage waste up to 50% in the goat's diet. Further, *Operculina turpethum* vines is a local plant and it can be added to goat diets by up to 35% without affecting gained weight daily (Dat et al., 2018). With supplementary food, rice distiller's by-product improved feed intake, feed conversion ratio and daily weight gain was reported when it was supplemented at 15% in dietary female Bach Thao goats (Truong and Trung, 2023). After mechanically extracting the coconut process from coconut meat, it is a coconut milk and coconut meat waste (CMW). The CMW contains low crude protein (approximately 5.81%), however high in ether extraction and carbohydrate content such as galactomannan and mannan in dry matter (Harnentis et al., 2022).

In this context of livestock farming economics, feed utilization for high income for farmers is very necessary. Goats can thrive in harsh environments and can utilize a wide range of forages (Nair et al., 2021). Furthermore, the goat population is estimated to have increased more than two-fold over the last decade in Vietnam (Don et al., 2023).

Based on literature review in databases, limited information is available for using coconut meat waste supplemented in Bach Thao goat. Therefore, the hypothesis of this study is that the coconut meat waste supplementation can affect the goat's feed intake, nutrient digestibility and nitrogen storage, and in overall efficacy of goat farming.

MATERIALS AND METHODS

The experiment was carried out, based on ethical regulations of animal studies in the farm of the Department of Animal Husbandry and Veterinary medicine of An Giang University, from January to April 2023.

The chemical composition of the experimental diets was analyzed in the laboratory of E205 (Ruminal animal production techniques – 4) of the Faculty of Animal Husbandry, Agriculture University of Can Tho University.

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Design of experimental

Four male Bach Thao goats (16.2±2.93 kg) used in this study. The processing methods (experimantal desing) are applied according to the Latin Square design (4x4) with the period of 2 weeks for adaptation and 1 week for data collection. The four treatments were supplement coconut meat waste at 0%, 5%, 10% and 15% dry matter. The coconut meat waste, Bach Thao goats and compositions of diets are shown in Figure 1 and 2 and also in Tables 1. The premix accounted for 2.0% of the feed supplement from rice distillers' by-products and coconut meat waste. The cabbage waste ratio for roughage was 35% of forages. The rice distillers' by-products, coconut meat waste, cabbage waste, and *Operculia turpethum* vines were purchased, harvested and cut down around Long Xuyen town, An Giang prefecture, Vietnam.

Figure 1 - Coconut meat waste preparation.

Figure 2 - Individual pen of goats in present experiment.

Table 1 - Ingredients used in the experiment.

Ingredients (% DM)	CMW0	CMW5	CMW10	CMW15
Coconut meat waste	0.00	5.00	10.0	15.0
Rice distillers' by-product	15.0	15.0	15.0	15.0
Cabbage waste	29.3	27.5	25.7	23.9
Operculina turpethum vines	54.4	51.1	47.8	44.5
Urea	1.00	1.00	1.00	1.00
Premix	0.30	0.40	0.50	0.60
Total	100	100	100	100

*CMW: coconut meat waste. CMW0, CMW5, CMW and CMW15: coconut meat waste at 0, 5, 10 and 15% per dry matter consumption.

The mixture content

Coconut residue, rice distillate, urea and premix were fed twice at 7:00 am and 1:00 pm. Cabbage was fed twice at 8:00 am and 2:00 pm. *Operculia turpethum* vines were provided freely.

Measurements taken

Feed, nutrients and energy intake

The provided feeds, feed wastes and feces were examined for dry matter (DM), organic matter (OM), crude protein (CP) and ash content following to AOAC (1990) procedures. However, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were investigated using the method of Van Soest et al. (1991). The metabolizable energy (ME) was determined following the method suggested by Bruinenberg et al. (2002), in which ME (MJ/animal/day) = 14.2 x DOM + 5.9 x DCP (with DOM/DCP<7.0 DOM is a digestible organic substance and DCP is able to be digested crude protein) of the diets or ME (MJ/head/day) = 15.1 x DOM (with DOM/DCP>7.0). Water intake was weighed before onset of feeding in the morning, daily. The amount of methane released was estimated according to the formula of Mills et al. (2003):

CH_4 (MJ/day) = 1.06 + 10.27 * roughage %) + 0.87 x DMI (kg/day).

- ***Apparent nutrient digestibility:*** Apparent digestibility of DM, OM, CP, NDF and ADF were determined according to McDonald et al. (2010). The experiment was conducted for four periods, and each period was 3 weeks including 2 weeks for adaptation and 1 week for samplings.

- ***Nitrogen retention:*** The nitrogen (N) content in feeds, waste, feces and urine was calculated following to the Kjeldahl method (AOAC, 1990). By eliminating the amount of N in feed residues, feces and urine from the N in the feed, the amount of N retained was determined, too.

- ***Daily weight gains (DWG):*** Experimental goats were weighed using an electronic scale and checked using live goat weights, which were weighed for 3 continual days in the early morning before feeding at the beginning and end of each experiment.

Statistical analysis

The data were analyzed using the ANOVA Linear Model (GLM) of Minitab Reference Manual Release 20.3 (Minitab, 2021). Differences among means were also compared by Tukey's test ($P<0.05$) when there was a significant overall effect. The statistical equation for this design was: $y_{ijk} = \mu + T_i + A_j + P_k + e_{ijk}$; where y_{ijk}: = the dependent variable, μ: the overall mean, T_i = the outcome of treatment (i = 1 to 4), A_j: the effect of Bach Thao goats (j = 1 to 4), P_k= the effect of period (j = 1 to 4), and e_{ijk} = the random error.

RESULTS AND DISCUSSION

Chemical composition of feeds

The results presented in Table 2 indicated that the NDF content was lower in rice distillers' by-product (28.3%) than coconut meat waste (57.5%), cabbage waste (33.1%) and *Operculina turpethum* vines (43.7%). Manh et al. (2009) reported that rice distillers' by-product CP and NDF were about 16.6-32.5% CP and 8.40-28.2% NDF. The nutrient of cabbage waste was 14.5%, 25.5% and 14.9% corresponding to CP, NDF, and ADF (Phong and Thu, 2018). The CP, NDF, and ADF of *Operculina turpethum* vines in the experimental goats same as that shown by Trung and Thu (2018) being 15.6% CP, 40.9% NDF and 11.2% ADF. Thus, the nutrient of feed in the present study agrees with previous studies.

Feed, nutrient and ME intakes of experimental goat

Results in Table 3 indicated that the DM consumption (g/head/day) was not different among treatments. It was 803, 861, 826 and 789 g corresponding to CMW0, CMW5, CMW and CMW15. However, the CMW5 and CMW10 treatments were higher DM intake than the CMW0 and CMW15 treatments. In detail, the DM/BW (%) increased from CMW0 to CMW5 and down to CMW10 and CMW15 treatments (3.34, 3.50, 3.46 and 3.28 %, respectively; Figure 3).

The DM/BW (%) in the present study was similar to the result reported by Hong et al. (2021) in Bach Thao goats was 2.72-3.01 %. The CP intake per day was not different among treatments. It was ranged from 147 to 167 g/head, which was similar to those reported by Phong and Thu (2018) being 149-159 kg/head/day. The NDF intake increased ($P>0.05$) by increasing coconut meat waste supplement levels in diets. It was 289, 322, 311 and 305 g/head/day corresponding to CMW0, CMW5, CMW and CMW15 treatments. It was explained by the high NDF of coconut meat waste supplement levels in diets. Metabolizable energy consumption was not different among treatments. While the highest value for CMW5 (9.19 MJ) compared to CMW10 (8.81 MJ), CMW15 (8.56 MJ) and the lowest value for CMW0 (8.39 MJ). The CH_4 (MJ/animal/day) was not different among treatments being 10.4-10.6 MJ.

In the present study, it's found that both DMI/BW, ME consumption and CH_4 product were not affected by supplementation with coconut meat waste. However, nutrient intake in CMW15 was lower than in CMW0, CMW10 and CMW5 treatments.

Apparent nutrient digestibility and digestive nutrient in the experimental goat

The result of Table 4 showed that the DM digestibility of CMW15 treatment (77.1%) was higher than CMW0 treatment (75.1%) but it was not different ($P>0.05$) compared to CMW5 and CMW10 treatments (76.4 and 75.9%, respectively). The DM digestibility in the experiment was higher than reported by Phong and Thu (2018), who found that DM digestibility from replacing increasing levels Para grass (*Brachiaria mutica*) with cabbage waste (*Brassica oleracea*) in the diets of Bach Thao goats about 64.9-66.6%. In our study, the CP digestibility was not different among treatments and it was 78.0, 78.3, 77.3 and 78.3% (CMW0, CMW5, CMW and CMW15, respectively).

Dong and Thu (2018) reported that CP digestibility of Bach Thao goats was about 75.6-77.8%. The NDF and ADF digestibility inceased by increasing the dietary coconut meat waste supplement levels and it were about 61.3-70.1% and 61.2-67.6%, respectively. Trung and Thu (2018) reported that NDF digestibility was about 56.2-59.6%. To our knowledge, coconut meat waste is qualitatively poor due to low crude protein and high dietary fiber (Harnentis et al., 2022). However, the feed in this study such as Rice distillers' by-product, Cabbage waste and *Operculina turpethum* vines were lower in fiber and higher in crude protein than coconut meat waste. Therefore, the matching and utilization of local feed were shown well for nutrient digestibility and digestive nutrients. Thus, increasing coconut meat waste intake from 5 to 15 %DMI was improved nutrient intake, digestibility and digestive nutrient.

Table 2 - Ingredient of feed (% basic DM) in the research

Feed \ Chemical composition	DM, %	OM, %	CP, %	NDF, %	ADF, %	Ash, %
Coconut meat waste	42.2	99.2	3.70	57.5	41.8	0.80
Rice distillers' by-product	11.2	97.0	27.2	28.3	18.5	3.00
Cabbage waste	8.36	88.6	16.3	33.1	23.3	11.4
Operculina turpethum vines	13.0	88.9	14.5	43.7	35.1	11.1
Urea	99.6	-	286	-	-	

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber

Table 3 - Effect of coconut meat waste on feed and nutrient intakes

Item \ Treatments	CMW0	CMW5	CMW10	CMW15	SEM	P-value
Feed intake, gDM/animal/day						
Coconut meat waste	0.00^{d}	40.4^{c}	82.5^{b}	118^{a}	5.340	0.001
Rice distillers' by-product	111	114	118	112	3.840	0.565
Cabbage waste	239^{a}	237^{a}	215^{ab}	189^{b}	7.480	0.010
Operculina turpethum vines	443^{a}	458^{a}	398^{ab}	357^{b}	14.50	0.010
Urea	7.71	8.02	8.22	7.85	0.227	0.475
Premix	2.31^{c}	3.20^{b}	4.10^{a}	4.69^{a}	0.152	0.001
Total nutrient intake, kg/animal/day						
DM	803	861	826	789	23.40	0.248
DM/BW, %	3.34	3.50	3.46	3.28	0.071	0.206
OM	711	767	739	710	20.80	0.272
CP	164	167	159	147	4.230	0.059
NDF	289	322	311	305	9.830	0.217
ADF	216	242	231	226	7.610	0.213
ME, MJ/day	8.39	9.19	8.81	8.56	0.338	0.433
Water intake, g/animal/day	63.9	69.8	64.0	67.1	5.290	0.838
Output, animal/day						
CH_4 (MJ/day)	10.5	10.6	10.5	10.4	0.047	0.175
Feces, gDM	201	201	197	178	7.990	0.224
Urine, g	4036^{a}	3899^{a}	3548^{ab}	3116^{b}	145.0	0.016

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: Metabolizable energy. CMW: coconut meat waste. CMW0, CMW5, CMW and CMW15: coconut meat waste at 0, 5, 10 and 15% per dry matter consumption

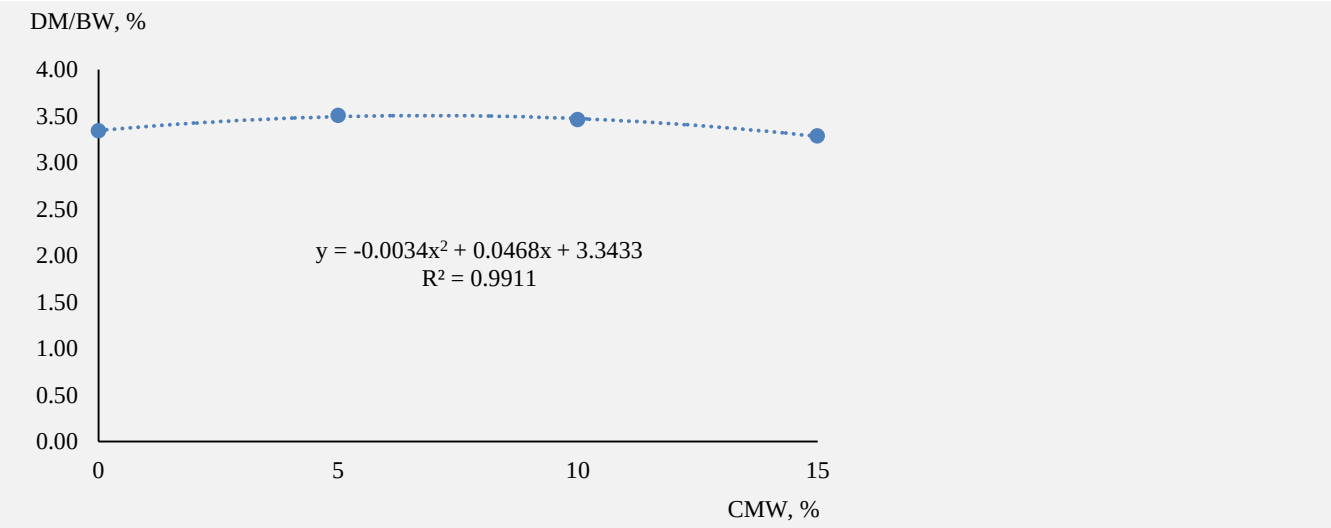

Figure 3 - DM intake per body weight is increased with a curvilinear trend as the feeding level of coconut meat waste was increased

Table 4 - The nutrient digestibility of goats in the study

Item \ Treatments	CMW0	CMW5	CMW10	CMW15	SEM	P-value
Nutrient digestibility, %						
DM	75.1	76.4	75.9	77.3	1.030	0.558
OM	75.8	77.1	76.7	78.1	1.030	0.525
CP	78.0	78.3	77.3	78.3	0.916	0.859
NDF	61.3	66.5	66.3	70.1	2.020	0.105
ADF	61.2	66.0	64.8	67.6	1.360	0.135
Digestive nutrient, g/animal/day						
DM	602	660	629	611	24.30	0.423
OM	538	593	569	555	22.00	0.416
CP	128	131	124	115	4.560	0.180
NDF	176	215	207	214	10.20	0.102
ADF	137	160	150	153	7.070	0.359

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber. CMW: coconut meat waste. CMW0, CMW5, CMW10 and CMW15: coconut meat waste at 0, 5, 10 and 15% per dry matter consumption

Storage nitrogen and gain weight daily

The N consumption of Bach Thao goats was similar (P>0.05) among treatments and ranged from 23.5 to 26.8 g/head/day. However, the nitrogen retention (g/head/day) had a trend of decrease by increasing dietary coconut meat waste. The daily weight gain in this study was similar (P>0.05) and had a trend of increase for CMW0, CMW5, CMW10 and CMW15 treatments being 180, 202, 194, and 187 g/head/day. The average weight gain (g/head/day) of the experiment was higher than other studies such as Phong and Thu (2018) being 71.4-116 g; Trung and Thu (2018) being 52.0-123g. In summary, results from this study suggest that DMI/BW (%) increased with increasing dietary CMW intake levels (5-10% DMI), while, the 15% CMW in the diet was lower than the CMW0 treatment. However, the ME intake was positive in Bach Thao goats the different coconut meat waste consumption levels. Although the N retentions did not affect this study. However, nutrient digestibility increased with increasing dietary coconut meat waste intake levels (5-15%DMI). Therefore, daily weight gain had a trend of increase in this study.

Table 5 - Storage nitrogen and gain weight daily of goat

Item \ Treatments	CMW0	CMW5	CMW10	CMW15	SEM	P-value
Nitrogen (N), g/head/day						
N intake	26.2	26.8	25.5	23.5	0.677	0.059
N fecal	5.78	5.75	5.69	5.07	0.182	0.092
N urin	11.1	11.7	10.2	9.43	0.854	0.336
N retention	9.67	9.15	7.29	9.01	1.400	0.670
N retention/$BW^{0.75}$	0.83	0.85	0.88	0.81	0.116	0.780
Body weight, kg						
BW Initial	22.5	22.3	22.6	22.2	0.314	0.780
BW Final	26.3	26.5	26.7	26.2	0.162	0.196
Daily weigh gain, g	180	202	194	187	12.80	0.667

CMW: coconut meat waste. CMW0, CMW5, CMW and CMW15: coconut meat waste at 0, 5, 10 and 15% per dry matter consumption.

CONCLUSION

Increasing coconut meat waste supplement levels in the diets of male Bach Thao goats improved feed and nutrient intake, nutrient digestibility, nitrogen storage, and daily weight gain. A level of 10% coconut meat waste in the diet could be recommended for further research in terms of available agricultural waste local utilization.

DECLARATIONS

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Corresponding author contributed on data analysis and the write up of the manuscript: Truong N.B. and Trung N.B. conceived and designed the experiments; Truong N.B. performed the experiments; Truong N.B. analyzed the data; Truong N.B. and Trung N.B. wrote the paper; all authors reviewed and approved the final manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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ASPIRATION, SLICING, AND FLUSHING MEDIUM TECHNIQUES IN COLLECTING OOCYTES OF SHEEP: SEARCHING FOR THE BEST METHOD

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ABSTRACT: The aim of this study was to compare the effect of the techniques of aspiration, slicing, and flushing medium in collecting oocytes on the quantity and quality of oocytes, the average time used for collecting oocytes per ovary, and the volume of the medium used. The material utilized was 274 ovaries from ewes aged 2.5 to 3.5 years and body weight ranging between 25 and 35 kg. This study used a Completely Randomized Design consists of 3 treatments of techniques were aspiration, slicing, and flushing medium. The parameters measured included the average quantity, quality, and weight of oocytes per ovary (right/left), the effect of the techniques of aspiration, slicing, and flushing medium on the quantity and quality of oocytes, and the efficiency of use of medium and time spent to collect oocytes by using aspiration, slicing, and flushing medium techniques. Data were analyzed by one-way analysis of variance. The results showed that the aspiration technique collected the highest percentage (P<0.01) of oocytes quality A (38.49%) compared to the slicing technique (17.93%), and the flushing medium technique (11.71%). In terms of time, the aspiration technique was the fastest (8-10 minutes) compared to the slicing technique (10-12 minutes), and flushing medium technique (13-15 minutes); meanwhile, the aspiration technique was the most efficient technique (1-2 ml) compared to slicing technique (3-5 ml) and flushing medium technique (6-10 ml). In conclusion, the aspiration technique is the best one for oocyte collection from sheep ovaries. This technique proves to be efficient in terms of quantity and quality of the oocytes collected, time to perform, and medium to use.

Keywords: Ewes, Ovaries, Oocytes, Reproductive techniques, Sheep breeding.

INTRODUCTION

In the biotechnology application on animal reproduction, in vitro embryo production requires good quality oocyte base materials. The oocytes in either the left or right ovaries can be collected using an appropriate technique and grown in an oocyte growth medium. In mammals, particularly sheep, the ovary may supply oocytes (primordial follicles) approximately 195,450-200,000 at birth (Sun et al., 2017; Cox and Yakov 2021). Yet only 475 oocytes reach their maturation stage and can be ovulated, while others live briefly and then die (Van Den Hurk, 2005). Some researchers found that the oocytes of cows cultured in an in vitro will still be able to grow and develop to the next stage (Ríos et al., 2015; Telfer et al., 2020). Similar result also found when they are cultured in a medium supplemented with cumulus cell culture (Bruynzeel et al., 1997), Fallopian tube epithelial cells (Barahona et al., 1997), gonadotropin hormone and steroid hormones (MacCallum et al., 1997; Lorenzo et al., 1997; Martínez et al., 2006). According to Sirard (2011), if the oocytes are incubated at a certain time, meiotic division may occur so that the oocytes will reach their optimal maturity level and are ready to be fertilized by spermatozoa. The zygotes formed can be grown into embryos until they reach a certain stage of growth (stages 2, 4, 8, 16, 32 or more than 32 cells). Thus, the ovaries potentially produce a good number of high-quality oocytes; however, collecting techniques used to accumulate the oocytes from the ovaries contribute their quality and quantity.

According to El-Sharawy et al. (2021), the flushing medium technique can collect an average of 4.32 oocytes from pubertal goats and 5.19 oocytes from adult goats. Wang et al. (2007) discovered that the slicing technique produces 6.3 oocytes, the flushing technique produces 5.8 oocytes, and the aspiration technique produces 2.9 oocytes per ovary. In addition, the finding of Martino et al. (1995) showed that slicing, aspiration, and flushing techniques produce an average of 1.71, 1.27, and 6.05 oocytes per ovary, respectively. Meanwhile, Hoque et al. (2011) identified that the technique of flushing, slicing, and aspiration is able to collect good quality oocytes per ovary on average 4.22, 4.14, and 3.28, respectively.

Regarding the review of the literature (Wani et al., 1999; Rodriguez et al., 2006; El-Sharawy et al., 2021), the number of oocytes collected using several techniques may have been considered in limited research-works.Therefore, further investigation in terms of collecting techniques for oocyte collection needs to be conducted. So, this study aimed to

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compare the effect of the techniques of oocyte aspiration, slicing, and flushing medium in collecting oocytes on the quantity and quality of oocytes, the average collection time used per ovary, and the volume of the medium used.

MATERIALS AND METHODS

Ethical regulations

The protocol of the current research was under the standard rule of animal treatment as designated in the Republic of Indonesia's law with number 41, 2014

Material

The materials used were 274 ovaries taken immediately from 137 ewes with range of age was 2.5 – 3.5 years and body weight of in between 25 and 35 kg. The chemical material used was sodium chloride, $CaCl^2$, de-ionized water, penicillin-streptomycin (SIGMA), and Kanamycin (SIGMA). The equipment used were dissecting microscope (Olympus SZ), inverted microscope (Olympus CK2) equipped with photographic equipment, laminar-flow hood (NUAIRE), filter with 0.22 µm (SIGMA) pore diameter, heating place (FISHER), petri dish (CORNING) with diameter 60 mm and 35 mm, disposable Pasteur pipette (VWR SCIENTIFIC), pipette-tip, Erlenmeyer, 25, 50 and 100 ml vial bottles, disposable syringes of various sizes 1, 5, and 10 ml, tweezers, scalpels, scissors, and gloves.

Oocytes collection

The total Oocytes have been collected from each technique was: 96, 88 and 90, respectively for aspiration, slicing and flushing medium techniques. This study used a Completely Randomized Design consists of 3 treatments of techniques were aspiration, slicing, and flushing medium. The aspiration technique was performed by sucking the follicular fluid having a diameter of 1.0 to 5.0 millimeters by using a 21 G syringe (Hashimoto et al., 1999) filled with 1.0 to 1.5 milliliters of Dulbecco's Phosphate Buffered Saline (D-PBS). The sucked follicular fluid was transferred into a petri dish, and then the quantity and quality of the oocytes were counted and observed, respectively using a 40 times magnification microscope. The slicing technique was performed by slicing the ovaries into small and thin parts using a scalpel in a petri dish containing D-PBS solution (Kouamo et al., 2014). The sliced parts were then transferred into another petri dish, counted, and observed for quantity and quality using a 40 times magnification microscope.

The flushing medium technique was done by puncturing repeatedly on the surface of the ovaries. Then rinsing them with the D-PBS medium slowly through punctures repeatedly across the entire surface of the ovaries using a syringe containing 1.0 to 1.5 milliliters of medium with a 21 G needle (Wongtra-ngan et al., 2010).

Parameters observed

Average quantity, quality, and weight of oocyte obtained per ovary (right/left). Effect of the techniques of oocyte collection on the quantity and quality of oocytes. The quantity and quality of the oocytes obtained by the techniques of aspiration, slicing, and flushing medium was statistically tested whether or not there were differences among the three techniques. The collected oocytes were classified according to their quality following Hoshino (2018) and Loos et al. (1992) that (1) Quality A, oocytes which are all surrounded by layers of cumulus cells, have homogeneous ooplasm and look clear and bright; (2) Quality B, oocytes which are mostly surrounded by cumulus cells, have homogeneous ooplasm but look rather dark on the edges; (3) Quality C, oocytes which are surrounded by a small portion of cumulus cells, have irregular ooplasm and dark; and (4) Quality D, oocytes with no cumulus cells around them, and the ooplasm looks very dark and irregular. Data related to time (minutes) needed to perform the techniques of aspiration, slicing, and flushing medium and the volume of collection medium used by all ovaries were tabulated averagely.

Statistical analysis

The length of time and the volume of the medium used reflected the efficiency of the best treatment. Collected data were analyzed by One-way Analysis of Variance with the techniques as independent variable.

RESULTS AND DISCUSSION

The results of the observation showed that the average weight of the right ovary (0.945 g) was heavier than that of the left ovary (0.855 g), the number of oocytes collected from the right ovary (11.7) was higher than that of the left ovary (10.1), and the quality A of oocytes collected from the right ovary (3.9) was better than that of the left ovary (2.2; Table 1). The number of oocytes according to the quality obtained by aspiration, slicing, and flushing medium techniques are presented in Table 2. The result showed that the aspiration technique collected the highest number of oocytes (1,351) from 96 ovaries, the flushing medium technique collected the second best (982) from 88 ovaries, and the slicing technique collected the lowest (853) from 90 ovaries.

Judging from the aspect of using the medium, the aspiration technique was more efficient than the slicing or flushing medium technique (Table 3). The results exhibited that the aspiration technique needs 1-2 ml per ovary, the slicing technique needs 3-5 ml per ovary, and the flushing medium technique needs 6-10 ml per ovary. This experiment showed that among the three treatments, aspiration techniques yielded the highest number of oocytes with quality A and B, respectively. Therefore, the quality of the oocytes by slicing and flushing techniques fell into categories C and D, respectively.

Table 1 - Average of oocytes quality collected from the right and left ovaries of sheep.

Ovary	Average weight of the ovary (gr)	Average oocyte collected per ovary according to its quality [1]				Number of Oocytes
		A	B	C	D	
Right	0.945	3.9^{ns}	3.3^{ns}	2^{ns}	2.5^{ns}	11.7
Left	0.855	2.2^{ns}	3.3^{ns}	2.1^{ns}	2.5^{ns}	10.1
Total	-	6.1	6.6	4.1	5.0	21.8

[2]Di [1]A: oocytes which are all surrounded by layers of cumulus cells, have homogeneous ooplasm and look clear and bright; B: oocytes which are mostly surrounded by cumulus cells, have homogeneous ooplasm but look rather dark on the edges; C: oocytes which are surrounded by a small portion of cumulus cells, have irregular ooplasm and dark; D: oocytes with no cumulus cells around them, and the ooplasm looks very dark and irregular. [2]NS=non-significant differences

Table 2 - The number of oocytes according to the quality obtained by aspiration, slicing, and flushing medium techniques

Oocyte Collection Techniques	Number of ovaries	Number and percentage of oocytes according to their quality [1]								Number of oocytes[2]
		A		B		C		D		
		Σ	%	Σ	%	Σ	%	Σ	%	
Aspiration	96	520	38.50	460	34.04	217	16.06	154	11.40	1351^{a}
Slicing	88	153	17.94	199	23.33	221	25.90	280	32.83	853^{b}
Flushing	90	115	11.71	226	23.01	284	28.92	357	36.36	982^{b}
Total	274	788	24.80	885	26.90	722	22.70	791	24.90	3186

[1]A: oocytes which are all surrounded by layers of cumulus cells, have homogeneous ooplasm and look clear and bright; B: oocytes which are mostly surrounded by cumulus cells, have homogeneous ooplasm but look rather dark on the edges; C: oocytes which are surrounded by a small portion of cumulus cells, have irregular ooplasm and dark; D: oocytes with no cumulus cells around them, and the ooplasm looks very dark and irregular. [2]Different superscripts in the same column show significant differences (P<005).

Table 3 - Efficiency among treatment of aspiration, slicing, and flushing medium in collecting oocytes according to quality, number of mediums, and time required per-ovary in average.

Collection Technique	Average number of oocytes according to quality [1] (per ovary)				Average volume of medium (ml)	Average time (minute)
	A	B	C	D		
Aspiration	5.4^{a}	5.2^{a}	2.5^{b}	1.9^{b}	1-2	8-10
Slicing	1.9^{b}	2.5^{b}	2.8^{b}	3.3^{ab}	3-5	10-12
Flushing	1.6^{b}	2.8^{b}	3.2^{a}	4.0^{a}	6-10	13-15
P-values	*	*	*	**		

[1]A: oocytes which are all surrounded by layers of cumulus cells, have homogeneous ooplasm and look clear and bright; B: oocytes which are mostly surrounded by cumulus cells, have homogeneous ooplasm but look rather dark on the edges; C: oocytes which are surrounded by a small portion of cumulus cells, have irregular ooplasm and dark; D: oocytes with no cumulus cells around them, and the ooplasm looks very dark and irregular. [2]Different superscripts in the same column show significant differences (*=P<0.05;**=P<0.01).

Data exhibited in Table 1 could be interpreted that the right and left ovary being were active. According to Hafez and Hafez (2000 because the right ovary naturally produces more gonadotropin hormone and participates in the oogenesis process, there are more mature oocytes. However, in terms of collection, the number of oocytes collected from the ovaries was also determined by the chosen technique of collection (Wang et al., 2007). The appropriate technique performed in collecting oocytes affects the number of good quality oocytes to be grown as embryos in vitro (Kruip et al., 1994).

The aspiration technique produced the highest percentages of oocytes with quality A (520; 38.50%) and B (460; 34.04%) while the slicing and flushing medium technique produced the highest percentages of oocytes with quality C (221; 25.90% and 284; 28.92%) and D (280; 32.83% and 357; 36.36), respectively. Meanwhile, the percentages of oocytes quality A (38.49%) and B (34.04%) collected by aspiration technique is higher than the average percentage of oocytes collected by the three techniques (22.50% and 26.79%), while the other two techniques collected less than the average. In comparison, the percentages of oocytes quality C and D collected by the slicing technique (25.90% and 32.82%) and the flushing medium technique (28.92% and 36.35%) is higher than the average percentages of oocytes

collected by the three techniques; oocytes quality C (23.62%) and oocytes quality D (26.85%), while the aspiration technique collected less than the average. Therefore, it could be assumed that the aspiration technique could be chosen as the best technique for collecting oocytes quality A and B. This finding is supported by the findings of previous studies, which stating that the aspiration technique in oocyte collection is more basic than other techniques (Marques et al., 2015).

The statistical analysis showed that there was a significant difference ($P < 0.01$) among treatments. Collecting oocytes using the aspiration technique was significantly different ($P<0.01$) compared to slicing and flushing medium techniques, as the last two techniques were not significantly different ($P>0.01$). This finding suggested that the aspiration technique significantly increased the number of oocytes collected. According to Gordon (1994), the total number of oocytes collected is strongly influenced by the oocyte separation technique from the ovary. The aspiration technique was able to collect oocytes precisely and directly on the spot, as the aspirator needle was easy to control and directed to the follicles (both large and small) that appeared on the surface of the ovary (Miller et al., 2004). In addition to the highest quantity and quality of the oocytes collected, this technique does not cause much oocyte damage (Machatkova et al., 1996).

The use of an aspirator needle is an essential factor in collecting good quality oocytes (Healy et al., 2015). In this study, a 21 G size needle was chosen because oocytes having a diameter of approximately 200 µm (Romão et al., 2010) within the ovaries were easily and precisely sucked by the needle of the aspirator. However, this finding was different from a previous study which reported that slicing technique produces more oocytes per ovary compared to the aspiration technique (Sofi et al., 2012). Yet, the aspiration technique performed was different from the one applied in this experiment. They used a vacuum pump aspiration technique to collect oocytes from sheep that had passed their mating season.

In this study, after being observed under a microscope, the number of oocytes collected by the slicing technique was small, as several oocytes were left in the ovary tissue which was caused by not being exposed to the slicing aimed at the follicle. As a result, the follicles with the oocytes remained intact. Moreover, in this technique, the slicing might damage the ovary when it hits the internal part of the follicles (Wang et al., 2007). As the structure of the cumulus cells enveloped the oocytes torn and decayed, the oocyte quality decreased (Lourenço et al., 2014; Xu et al., 2015). A similar result did happen with the flushing medium technique. As the needle randomly punctured the ovaries causing irregular holes, the oocytes were difficult to detach from the follicle when the medium was injected into the ovary. Consequently, the severe damage dropped dramatically in the quantity and quality of the oocytes collected.

The aspiration technique needed 10 minutes, as the position of the follicles was very clearly visible on the ovary surface; therefore, it was easy and fast in conducting aspiration. The slicing technique needed 10-12 minutes because it took time to make ovarian slicing searching for oocytes. Meanwhile, the flushing technique needed 12-15 minutes since puncturing to the ovaries had to be done first followed by 2-3 times rinsing for ovary searching. In terms of time, the aspiration technique was faster than the other two techniques. The findings of this investigation supported those of Martínez et al. (2006), who found that the aspiration approach for oocyte collecting is quick, simple, and effective. Data and analysis previously discussed suggested that the implementation of the aspiration technique was better than that of the other two techniques, as the aspiration technique was able to collect a high quantity and quality of oocytes, was efficient in using the medium and was fast in terms of time.

CONCLUSION

Among the three techniques, the aspiration technique is the best one for oocyte collection from sheep ovaries. This technique proves to be efficient in terms of quantity and quality of the oocytes collected, time to perform, and medium to use. Therefore, this technique is expected to help develop the effort of increasing embryo production in vitro.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

YS Ondho and Sutiyono: Idea and research design; ET Setiatin, S Sutopo, and D Samsudewa: Data collection; E Kurnianto, and A Setiaji: Data analysis and Writing the manuscript; DA Lestari: Writing the manuscript

Conflict of interests

The authors declare that they have no conflict of interest.

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EFFECT OF REPLACING CORN WITH THREE FIBRE SOURCES ON GROWTH PERFORMANCE AND CARCASS QUALITY OF BROILER CHICKEN

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RESEARCH ARTICLE

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ABSTRACT: The experiment was carried out to evaluate the effect of replacing corn with three dietary fibre sources on the growth performance and carcass quality of broiler chicken. One hundred and forty four one-day old broiler chickens were used for the experiment. The birds were brooded for two weeks on commercial starter diet after which they were subjected to four experimental diets or treatments. The birds were randomly distributed into four treatments with three replicates, each replicate consisting of 12 birds. All birds in each treatment were fed with different–diet and weighed at the end of every week. The experimental design used was a completely randomized design. The experimental treatments were designated as treatment T1, T2, T3, T4 while T1 was tagged as a control diet without any source of dietary fibre, T2 as a diet with wheat bran as a source of dietary fibre, T3 as a diet with rice bran as a source of dietary fibre, T4 as a diet with brewers dried grain (BDG) as a source of dietary fibre. There was no significant ($p>0.05$) difference in the initial weight of the birds across the treatments, but there was a significant ($p<0.05$) difference in the final weight and body weight gain of the birds where T2 had the highest body weight gain and T3 has the lowest body weight gain. This trend was also observed in the carcass. Based on the result of this experiment wheat bran can be used as a source of dietary fibre for better growth performance of broiler chicken at a low inclusion level.

Keywords: Broiler, Carcass, Corn, Dietary fibre, Growth performance.

INTRODUCTION

Broilers are domesticated chicken usually raised for meat purpose, broilers have the genetic potential to gain a significant amount of weight for a very short period of time, in order to achieve this, nutrition of the birds has to be taken very important and be closely monitored. Feed is one of the most important factors which impacts broiler performance (Ferket and Gernat, 2006). Broilers consume a large amount of feed. Feed costs vary with the cost of ingredients, but normally feed costs for broilers represent 65-85% of the variable cost of production of the live broiler (Da Costa et al., 2017). This cost has risen significantly in the past few years due to increases in prices of many feed ingredients, particularly cereal grains, which are used mainly as a source of energy for the birds (Donohue and Cunningham, 2009). Total feed costs can be reduced by the inclusion of less grain in the diet (Bikker and Jansman, 2023; Sibanda et al., 2023). Such a practice would also improve the quality of poultry meat, as a result of the very rapid growth of birds and over-consumption of very high-quality feed (Azizi et al., 2011; Baéza et al., 2022). Researches have been done to check the nutrition requirement and how feed ingredients such as dietary fibre source can be effectively used for the broilers to be able to maximize their genetic potential.

Dietary fiber can be said to be a part of plant material consisting mainly of cellulose and non-cellulosic polysaccharides and a non-carbohydrate component lignin. These components are highly resistant to hydrolysis by the alimentary enzymes and cannot, therefore be digested or absorbed in the blood stream. Yet fiber plays an important role in poultry diets, if applied properly. Dietary fibers are diverse in chemical composition and can be grouped generally by their solubility, viscosity and ferment ability, fiber can be sub divided into two main components: soluble fiber and insoluble fiber. Dietary fiber is usually considered as energy diluents and as an anti-nutritional factor which negatively affects feed consumption and nutrient digestibility which may later have negative influence on the growth performance, in commercial poultry diet formulation. However, there are some reports on the favorite effects of moderate dosage of dietary fiber in the diet which can improve growth performance traits of broilers (Adibmoradi et al., 2016; Sekh and Karki, 2022). Broilers growth performance can be assess using some of the types of dietary fiber that has been frequently mentioned; Wheat bran, rice bran, and brewers dried grain (BDG). Inclusion of dietary fiber in the diet of broiler chicken is very low as part of nutrient requirement due to the fibrous component in the feed materials and also the presence of anti-nutritional factor in the feed materials can have negative effects on the growth performance of broiler chicken (Swennen et al., 2010). Therefore, the study was carried out to evaluate the effects of replacing corn with three fibre sources on the growth performance and carcass quality of Abor Acre broiler chickens.

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MATERIALS AND METHODS

Ethical Approval

The experiment was approved by the Federal University of Oye Ekiti (FUOYE) Faculty of Science Research Ethics Committee (RECOM), and reviewed and considered the submitted research protocol and hereby gives ethical approval (FUOYEFSC 201122-REC2022/014) to carry out the research.

Experimental location

The experiment was conducted at the Teaching and Research Farm of the Department of Animal Production and Health, Faculty of Agriculture, Federal University Oye-Ekiti, Ekiti State, Nigeria. The location is within longitude 5.5145^{0}E and latitude 7.7983^{0}N and at an elevation of 570m above sea level. The climate of the study area is typically tropical with relative humidity ranging from 57-92% and a mean average daily temperature of 68-90^{0}F.

Experimental birds and management

A total number of 144 day old Abhor acre birds was purchased from CHI farms, Ibadan, Oyo state Nigeria and were in a pen with concrete floors, walls and galvanized roof with open sided walls. Washing, cleaning and disinfection of the pen was done five days prior to the arrival of the birds, wood shavings were used as bedding materials for the birds, in other to absorb fecal, water and to prevent coccidiosis and the bedding was changed twice weekly and if water spillage is noticed, the bedding was changed immediately. The chicken was raised on deep litter system throughout the experimental period. Coal pot with charcoal and electric bulbs were used as source of heat for the chicks during the brooding period. Vaccine against Infectious Bursal disease (using Attenuated Infectious Bursal Disease Vaccine) was given one week (day 7) after housing and on the third week (day 21) of life respectively. Vaccine against Newcastle disease using (lasota vaccine) was given on the second week (day 14) and fourth week (Day28) of life respectively. All vaccines were administered orally. Antibiotics and vitamins were administered to the birds except the days of vaccination. The birds were fed ad-libitum with broiler starter diet for three weeks using the experimental diet for each treatment and broiler finisher for three weeks. Water was given ad-libitum throughout the experiment.

Experimental design and diets

There were total number of four treatments and three replicates per treatment with a total number of 12 birds per replicate in a completely randomized design. All chicks were fed commercial feed for the first two weeks of life, after which they were fed with experimental diets. Formulated experimental broiler starter diets were given two weeks after brooding from the 3rd week to the 4th week while the broiler finisher diets were given for the rest 4weeks (5th to 8thweek). The experiment lasted for eight weeks. The experimental diet was formulated for both starter (Table1) and finisher phase (Table 2). The major source of energy for the diet was maize and the major source of crude protein was soya bean meal. The control diet T1 was feed formulated without dietary fibre, T2 was feed formulated with wheat bran (6%) as the source of fibre, T3 was feed formulated with rice bran (6%) as the source of fibre, and T4 was feed formulated with brewers dried grain (BDG) (6%) as the source of fibre.

Table 1 - Ingredients and Nutrient composition of experimental diets fed to broiler starter chicken (3–4weeks)

Ingredients	T1 (Control %)	T2 (6% WB)	T3 (6% RB)	T4 (6% BDG)
Maize	53.65	48.65	48.65	48.65
Soya bean	40.00	39.00	39.00	39.00
Wheat bran	0.00	6.00	0.00	0.00
Rice bran	0.00	0.00	6.00	0.00
B D G	0.00	0.00	0.00	6.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Premix	0.10	0.10	0.10	0.10
Toxin binder	0.05	0.05	0.05	0.05
TOTAL	100.00	100.00	100.00	100.00
*ME				
Determined				
Moisture (%)	9.48	9.42	9.80	9.15
CP (%)	25.38	23.76	22.70	21.80
CF (%)	6.30	7.15	8.92	5.80
EE (%)	4.08	4.16	3.18	3.78
Ash (%)	10.79	9.57	9.24	11.91
NFE (%)	43.97	45.94	46.16	47.56

*ME=Metabolizable energy (Calculated); CP=Crude Protein; CF=Crude Fibre; EE=Ether Extract; NFE=Nitrogen Free Extract; TI: Treatment 1 (diet without dietary fiber), T2: Treatment 2 (diet with wheat bran as source of fiber), T3: Treatment 3 (diet with rice bran as source of fiber), T4: Treatment 4 (diet with BDG as source of fiber), BDG: (brewer's dried grain).

Table 2 - Ingredients and Nutrient composition of experimental diets fed to broiler finisher chickens (5-8 weeks)

Ingredients	T1 (Control %)	T2 (6% WB)	T3 (6% RB)	T4 (6% BDG)
Maize	58.65	53.65	53.65	53.65
Soya bean	35.00	32.00	32.00	32.00
Wheat bran	0.00	8.00	0.00	0.00
Rice bran	0.00	0.00	8.00	0.00
B D G	0.00	0.00	0.00	8.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Premix	0.10	0.10	0.10	0.10
Toxin binder	0.05	0.05	0.05	0.05
TOTAL	100.00	100.00	100.00	100.00
*ME				
Determined				
Moisture (%)	10.89	10.50	10.58	10.33
C.P (%)	22.72	26.80	24.80	24.50
C.F (%)	5.38	6.10	7.51	6.33
E.E (%)	4.30	4.70	4.58	4.09
Ash (%)	6.90	6.88	7.58	9.95
NFE (%)	49.81	47.02	44.95	44.80

*ME= Metabolizable energy (Calculated); CP=; CF=; EE=; NFE=; TI: Treatment 1 (diet without dietary fiber), T2: Treatment 2 (diet with wheat bran as source of fiber), T3: Treatment 3 (diet with rice bran as source of fiber), T4: Treatment 4 (diet with BDG as source of fiber), BDG: (brewer's dried grain).

Data collection

Parameters determine were initial live weight, final live weight, average weight gain, total weight gain, feed intake and feed conversion ratio, carcass weight and organ weights.

Statistical analysis

The data collected were subjected to the analysis of variance procedure (ANOVA) according to (SAS v9.2, 2012) at $p>0.05$. Significant means was separated using Turkey's honestly significant difference.

Statistical model: Yij=U + Di + eij

Yij–General Observation; U–General Mean; Di—Effect of the dietary treatment; Eij—Random residual error

RESULTS

The result of the proximate analysis of the formulated starter and finisher diet as presented in Tables 1 and 2 indicates that, for the starter diet T1 (control diet) has the highest CP (25.38%) and T4 (diet with BDG) has the lowest CP (21.80%) while for the finisher diet T2 (diet with wheat bran) has the highest CP (26.80%) and T1 (control diet) has the lowest CP (22.72%). The CF (crude fibre) is higher for T3 (8.92%), (Diet with rice bran) for the starter diet and it is lower for T4 (5.80%) (Diet with BDG) while for the finisher diet T3 (diet with rice bran) has the highest CF (7.51%) and T1 has the lowest CF (5.38%).

Growth performance of broiler chicken fed three different dietary fibre sources

Table 3 shows the effect of three different dietary fibre sources on the growth performance of broiler chicken. There was no significant difference ($P>0.05$) among the birds across the treatments for initial weight at two weeks, but the birds in T3 (diet with rice bran) have the highest initial weight (219.13) and the birds in T1 (control diet) has the lowest initial weight (208.27). There was significant difference ($P<0.05$) among the birds across the treatments for final weight, with birds in T2 (diet with wheat bran) having the highest final weight (2268.23) and the birds in T3 (diet with rice bran) having the lowest final weight (2110.87). Birds in T1, T2 and T4 have similar weight. Average weight gain for birds in T2 (diet with wheat bran) was significantly ($p<0.05$) greater (47.769) while T3 (diet with rice bran) was significantly ($p<0.05$) lesser (43.98). The same trend was observed in Total weight gain with birds in T2 (diet with wheat bran) significantly ($p<0.05$) having greater weight gain (2055.21) while T3 (diet with rice bran) significantly ($p<0.05$) having lesser weight gain

(1891.14). There was no significant difference (P>0.05) among the birds across the treatments for Total Feed Intake, but birds in T4 (diet with BDG) consumed the highest feed (5999.6) and the birds in T1 (control diet) consumed the lowest feed (5500). There was no significant difference (P>0.05) among the birds across the treatments for FCR, but the bird in T4 (diet with BDG) has the highest FCR (3.0340) and the bird in T2 (diet with wheat bran) has the lowest FCR (2.7015).

Carcass weight of broiler chicken fed different fibre diet

Table 4 shows the effect of different dietary fibre on carcass weight of broiler chicken.

Result shows that there were significant differences in the live weight, slaughter weight, dress weight, wing weight, thigh weight, drum stick weight, breast weight and neck weight. From the table, T2 recorded the highest value in all the parameters measured in treatments across the groups except for the wing weight, with T1 (diet without dietary fibre) having the highest (101.25) wing weight and T3 (diet with rice bran as source of fibre) having the lowest (89.03). For internal organs, result shows that there were significant differences (P<0.05) in the weights of the intestine, crop, bile, pancreas and empty gizzard. It was observed that T2 (diet with wheat bran as source of fibre) had the highest values in all the parameters measured and T3 (diet with rice bran as source of fibre) recording the lowest.

Table 3 - Growth performance of broiler chicken fed different sources of dietary fibre

Parameters	T1	T2	T3	T4	SEM	LOS
Initial weight(g)	208.27	213.02	219.13	203.30	5.3219	NS
Final weight(g)	2225.40^{ab}	2268.23^{a}	2110.87^{b}	2181.20^{ab}	21.5620	*
Avg. weight gain(g)	46.91^{ab}	47.769^{a}	43.98^{b}	45.998^{ab}	0.4637	*
Total Weight gain (g)	2017.13^{ab}	2055.21^{a}	1891.14^{b}	1977.90^{ab}	19.9412	*
Total Feed Intake (g)	5500.00	5552.20	5597.10	5999.60	141.9530	NS
FCR	2.7286	2.7015	2.9612	3.0340	0.0713	NS

^{ab} Mean with different superscripts in the same row are significantly different (P<0.05). TI: Treatment 1 (diet without dietary fiber), T2: Treatment 2 (diet with wheat bran as source of fiber), T3: Treatment 3 (diet with rice bran as source of fiber), T4: Treatment 4 (diet with BDG as source of fiber), BDG: (brewer's dried grain). LOS: (Level of Significance) FCR: (Feed Conversion Ratio), Avg: (Average), NS: not significant at (P>0.05) level of Significant.

Table 4 - Weight of carcass (primal cuts) and internal organs of broiler chickens fed different fibre diets for 43 days (6 weeks).

Parameters (g)	T1	T2	T3	T4	SEM	LOS
Live weight	2225.40^{a}	2268.23^{a}	2110.87^{b}	2181.20^{ab}	85.21	*
SW	2156.40^{a}	2192.73^{a}	2033.87^{b}	2100.95^{ab}	93.01	*
DW	1715.64^{a}	1752.00^{a}	1581.87^{b}	1639.20^{b}	60.06	*
Dressed	77.09	77.24	74.94	75.15	2.97	NS
Wing weight	101.25^{a}	98.73^{b}	89.03^{b}	93.16^{b}	8.73	*
Thigh weight	283.98^{a}	287.42^{a}	268.05^{b}	273.71^{b}	4.01	*
DSW	195.23^{a}	198.45^{a}	184.31^{b}	185.18^{b}	6.07	*
Back weight	296.13	299.57	278.73	289.20	11.38	NS
Breast weight	496.15^{a}	501.10^{a}	460.66^{b}	461.74^{b}	20.21	*
Neck weight	64.10^{a}	65.05^{a}	54.63^{b}	60.33^{ab}	7.63	*
Shank weight	47.00	48.26	39.37	41.89	9.21	NS
Head weight	55.25	56.70	50.47	54.53	7.02	NS
Internal organs(g)						
Liver	30.56	34.89	28.72	30.01	6.23	NS
Spleen	1.80	1.81	1.78	1.80	0.20	NS
Lung	8.98	9.01	8.51	8.99	0.68	NS
Heart	7.91	7.97	7.09	7.95	0.90	NS
Intestine	64.58^{ab}	72.81^{a}	55.50^{b}	64.76^{ab}	9.00	*
Kidney	1.21	1.22	1.19	1.22	0.06	NS
Crop	12.21^{a}	12.30^{a}	11.37^{b}	12.19^{a}	0.64	*
Bile	2.03^{a}	2.04^{a}	1.82^{b}	2.01^{a}	0.19	*
Pancreas	4.51^{a}	4.57^{a}	4.33^{b}	4.53^{a}	0.13	*
Empty Gizzard	42.76^{ab}	50.10^{a}	36.31^{b}	46.00^{ab}	4.21	*

^{ab} Mean with different superscripts are significantly different at (P<0.05). TI: Treatment 1 (diet without dietary fibre), T2: Treatment2 (diet with wheat bran as source of fibre), T3: Treatment3 (diet with rice bran as source of fibre), T4:Treatment4 (diet with BDG as source of fibre), BDG: (brewer's dried grain). LOS=Level of Significance; SW=Slaughter weight, DW =Dress weight, DSW=Drum stick weight.

DISCUSSION

Growth performance of broiler chicken fed different sources of dietary fibre

The result of the growth study in table 5 shows that there was no significant difference (P>0.05) among the growth parameters like initial weight, total feed consumed and FCR in the birds across the treatments. It was observed that the birds in T4 (diet with BDG) consumed more feed than all other treatments and they have the highest FCR, which means they do not convert the feed they consumed into an appreciable body weight when compared to the other treatments. However, the birds in T1 (diet without dietary fibre) had the least feed consumption and they have an appreciable FCR which is an indication that they were able to convert feed consumed into appreciable body weight, T2 has a moderate consumption rate and the lowest FCR which is an indication that they were able to convert a good amount of feed they consumed into body weight excellently and they have the highest weight gain (2055.21g).

The result of the growth study also shows that there is a significant difference (p>0.05) in the total weight gain and average weight gain between T1 (control diet) and T4 (diet with BDG), this can be due to the difference in the crude protein level of the diet for both starter and the finisher diet. T2 (diet with wheat bran) has the highest body weight gain 2055.21g and the highest average body weight gain 47.769g among all the treatments and it was observed that T2 (diet with wheat bran) was superior in converting feed into weight gain than birds in other treatments. Courtin et al. (2008) and Craeyveld et al., (2010) recorded that inclusion of moderate level of wheat bran improves growth performance in broiler chicken.

Broiler chickens fed rice bran has the lowest weight gain, this is in correlation with Gallinger et al., (2004) which says the inclusion of rice bran can bring about poor growth especially at a higher percentage. The lignocellulosic bond in rice is such that the monogastric animals i.e., broilers cannot break it down for release of glucose for muscle building. Also, the crude protein value is lower in rice bran compared to the other fibre diets. It is worth noting that rice bran has a low glycemic index compared to other fibre diets like wheat bran and brewer's dry grain. The result of the study also showed that broiler chickens fed control diet performed moderately but not better than those fed wheat bran diet which can be due to low level of feed intake, feed intake can be improve by addition of reasonable source of fibre as reported by Gonzalez et al. (2010). Broiler chickens fed BDG has the least performance but they have the highest feed consumption among other treatments, this can be due to the presence of high lignin and non-starchy polysaccharides which act as anti-nutritional factor as reported by Karlsen and Skov (2022). The live weight obtained for broilers in this study were lower than the value (2480±9.75g) reported for broilers by Omojola and Fagbuaro (2005).

Carcass weight and internal organs of broiler chicken fed different sources of dietary fibre

Lu et al (1996) reported that relative organ weight could be used as an indicator of organ function.

In the present study, the experimental diet with the highest (7.51%) Crude Fibre (T3 Diet with rice bran) significantly decreased weight of carcass parameters of broiler chicken fed different fibre diets for 43 days (6 weeks) on the following parameters measured; Live weight, dressed weight, wing weight, thigh weight, drum stick, back weight, breast weight and neck weight of broiler chickens compared with the control and other treatment groups. Similarly, Shahin and AbdElazeem (2005) found that fibre inclusion in broiler diets decrease carcass weight.

Also, in this study reduced (6.10%) dietary fibre increased the weight of the intestine in treatment 2 and in the weight of internal organs of broiler chicken fed different fibre diets (72.81g) (Table 6) which is contrary to the findings of Mateos et al (2012) who reported that dietary fibre decreased the intestinal length and weight of the organs of broiler chicken. Consequently, these changes might reduce carcass yield (Jorgensen et al., 1996; Tejeda and Kim, 2021). Present result is in agreement with a few others (Preston et al., 2000; Taylor and Jones, 2001; Mourao et al., 2008) in which dietary fibre increased the relative length and weight of the small intestine. The longer relative length of the small intestine in the fibre groups might be due to the increased effort of this organ to adapt to improve feed consumption and nutrient uptake (Mourao et al., 2008; Sittiya et al., 2020). However, the results of our study did not agree with those of a few other reviews. For instance, Amerah et al. (2009) and Sklan et al. (2003) found that increasing the insoluble fiber in the diet reduced the length of the small intestine. These conflicting findings may be due to the differences in the physiochemical characteristics, feed interactions and amount of the fibre sources as well as particle size (Mateos et al., 2012).

The decrease in the carcass or primal cuts and organs can be related to the bird's live weight that followed the same trend across treatments. These organs could be proportional to their respective live weights as may be fixed by their genetic makeup since the experimental birds are of the same breed (Abor Acre).

CONCLUSION

Based on the result of this experiment, birds fed with wheat bran had the highest live weight and carcass parameters or primal cuts as well as internal organs or offal's with no adverse effect. Wheat bran is also affordable and available since it is widely distributed throughout the tropics. However, a little percentage of inclusion of wheat bran (because of its high fibre content) will help improve the performance of broilers. Wheat bran is hereby recommended to intending poultry

farmers as a source of dietary fibre in the feed of broiler chicken at a little percentage (8%) for better growth performance of broiler chicken.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Mercy Oluwaseyi Okowonleyin collected the samples and carried out the field work and wrote the first draft. Anthony Henry Ekeocha supervised the overall research, Ademiju Adeolu AGANGA provided the resources for the research work, Oloriire Kolade Aderemi assisted in the statistical analysis and Patrick Chinedu Emerue revised the draft of the manuscript. All authors approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare no competing interests in this research and publication.

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PCR-BASED STUDY ON VIRAL PATHOGENS CIRCULATION AMONG CERVIDS IN THE MOSCOW REGION

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ABSTRACT: A molecular survey of selected viruses in free-ranging cervids was conducted in 15 different districts of Moscow region. Samples were collected from 178 game animals including 144 moose (*Alces alces*), 19 roe deer (*Capreolus capreolus*) and 15 deer without species information. Nasal swabs and tissue samples including parts of the nasal septum, upper tracheal rings, lung, heart, liver, kidneys and pooled organ samples were tested using polymerase chain reaction (PCR). Samples were studied for pestiviruses, herpesviruses, coronaviruses, group A rotaviruses, adenoviruses, hepatitis e and parainfluenza type 3 virus. None of the samples were positive for Bovine Coronavirus and SARS-COV-2, hepatitis E virus and parainfluenza type 3 virus. PCR results were positive for bovine herpesviruses (5.05%), pestiviruses (0.56%), rotaviruses (1.68%). DNA of a new adenovirus, presumably causing a mild course of animal respiratory disease, was detected in samples of 6 animals (3.37%). In conclusion, the conducted studies have shown that game animals of the Moscow region can be a natural reservoir of cattle viruses, and this must be taken into account when planning and organizing measures for the control and eradication of such notifiable diseases as bovine viral diarrhoea and infectious bovine rhinotracheitis. Monitoring studies and general disease surveillance of wild animal populations provide additional information on the epidemiology of infectious diseases in the region and allow timely measures to be taken to protect wild animals, domestic animals and the public.

Keywords: Cattle, Deer, Epidemiology, PCR, Viral infection.

INTRODUCTION

Domestic cattle (*Bos taurus taurus*) belong to the same superfamily Cervoidea as cervids (Fernández and Vrba, 2005; Kuznetsova et al., 2005). Also, a quantity of cervids inhabits the forests of the European part of the Russian Federation (Rumyantsev et al., 2018). Animals may enter public gardens, city parks, or highways, where the likelihood of them coming into contact with people increases. Infected ungulates entering livestock pasturelands pose a separate danger. In this case, there can introduce infectious diseases into livestock farms (Böhm et al., 2007; Yatsentyuk et al., 2022).

Scientific studies indicated that wild ruminant ungulates can carry viruses that are common to wild and farmed animals and also those that are dangerous to humans (Ros and Belák, 1999; Ricci et al., 2019; Althof et al., 2023; Feng et al., 2023).

Viruses from different groups can either cause high mortality or cause subclinical forms of infection in deer (Auer et al. 2022; Domshy et al. 2023; Feng et al., 2023). Recently, wild animals have also been considered as potential sources or reservoirs of new viral pathogens, where viruses can persist and change, and can be transmitted to farm animals (Cripps et al. 2019; Yatsentyuk et al., 2022; Feng et al., 2023).

Herpesviruses are the viral group mostly studied on wild ungulates, and in this field, representatives of Alphaherpesvirinae are studied more frequently (Ros and Belák 1999; Rola et al. 2017). However, the presence of closely related α-herpesviruses that can occur in ruminants makes it difficult to estimate the prevalence by serological methods (Besi et al., 2018; Bianchessi et al., 2022). Bovine herpesviruses type 1 (BoHV-1) and type 5 (BoHV-5), buffalo herpesvirus type 1 (BuHV-1), caprine herpesvirus type 1 (CpHV-1), deer herpesvirus type 1 (CvHV-1), deer herpesvirus type 2 (CvHV-2) and elk herpesvirus type 1 (ElkHV-1) can induce similar antibodies in animals (Thiry et al., 2006). This may cause misinterpretation of test results. The prevalence of another herpesviral group - Gammaherpesvirinae, including bovine herpesvirus types 4 (BoHV-4) and 6 (BoHV-6), elk gammaherpesvirus (Elk-LHV) and fallow deer lymphotropic herpesvirus (LHV) in cervids is almost not studied, widely (Kálmán and Egyed 2005; Yatsentyuk et al., 2022).

Pestiviruses are the viral group that is an interesting object of research; Bovine viral diarrhea virus (BVDV), which causes the OIE-notified viral diarrhea disease, is widespread throughout the world (Scharnböck et al., 2018). BVDV is

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often detected in Russia in cattle (Glotov et al., 2016 a, b). The prevalence of this virus in wild ruminants in Russia is not well documented (Pchelnikov et al., 2023).

Some researchers believe that wild ruminants become infected exclusively from livestock, and not vice versa (Fernández-Aguilar et al., 2016). Others have suggested that cervids are a reservoir for BVDV (Rodríguez-Prieto et al. 2016). Bovine coronavirus (BCoV) and parainfluenza virus-3 (PIV-3) are also quite common among cattle in different countries (Burimuah et al., 2020; Vlasova and Saif, 2021). Coronavirus studies conducted in Canada, USA, Japan and South Korea have identified bovine-like CoVs in 6 deer species (Amer, 2018), and in recent years, information has appeared about the identification of another coronavirus in deer - SARS-COV-2, which caused the COVID-19 pandemic (Feng et al., 2023).

Parainfluenza virus-3 (PIV-3) is also can infect different species of ungulates. It has been detected in camels, buffaloes and different deer. PIV-3 is thought to increase an animal susceptibility to other respiratory pathogens. Moreover, PIV-3 itself can either cause subclinical or cause acute manifestations in animals. Serological and PCR studies confirm the prevalence of this respiratory infection in livestock. But reports of detection of PIV-3 in wild animals are few (Dastjerdi et al., 2022). There are even less reports of Bovine respiratory syncytial virus (BRSV) circulating in wild ungulates (Bergmann et al., 1990).

Rotavirus infections caused by group A rotaviruses (RVA) are a problem in young cattle (Steele et al. 2004). RVA are widespread in many countries, but information on the occurrence of rotaviruses in wild ungulates has only begun to accumulate in recent years. The viruses were found in cervids in Germany, Slovenia, and South Korea (Althof et al., 2023). The researches note that the relationship of animal RVA strains with human strains indicates the zoonotic potential of RVA and requires study (Jamnikar-Ciglenecki et al., 2016; Althof et al., 2023).

Hepatitis E virus (HEV) is a zoonotic virus that can also be detected in wild ungulates. HEV RNA was detected in wild boars and different deer samples from Lithuania (Spancerniene et al., 2018), Germany (Anheyer-Behmenburg et al., 2017), Spain (Boadella et al., 2010), Japan (Takahashi et al., 2022).

Adenoviruses are a group of viral pathogens that are often found in various vertebrates. Mastadenoviruses and Atadenoviruses are pathogens of various ungulates, occurring in cattle and sheep, as well as in various cervids. Different adenoviruses were described in wild ungulate populations. White-tailed deer (*Odocoileus virginiana*), red deer (*Cervus canadensis nelsoni*), mule deer (*Odocoileus hemionus*), and moose (*Alces alces*) have been shown to be susceptible to the Atadenovirus Odocoileus adenovirus 1, which causes adenoviral hemorrhagic disease with high mortality (Kauffman et al., 2021). Mastadenovirus Odocoileus adenovirus 2, isolated in 2017 from white-tailed deer, unlike Odocoileus adenovirus 1, does not cause an acute course of the disease and epizootic outbreaks. It is manifesting in mild respiratory lesions and slight thinning of immune tissues (Ridpath et al., 2017).

The purpose of this study was to find out whether there is data on the presence of viruses of different groups in the population of free-living cervids in the Moscow region using molecular methods. The first goal of the study was to screen samples for bovine viruses common to cattle and cervids, including bovine herpes viruses, bovine coronavirus, bovine viral diarrhea virus, and parainfluenza virus-3. The second objective was to assess the presence of viruses with zoonotic potential - hepatitis E virus, rotavirus and SARS-COV-2. The third task is to investigate the presence of viruses specific to cervids.

MATERIALS AND METHODS

Ethical approval

This study used samples obtained from hunted animals. All licences for hunting were issued by the Ministry of Ecology and Natural Resources of the Moscow Region. The study protocol was approved by the Ethics Committee of the Federal State Budgetary Institution “All-Russian State Center for Quality and Standardization of Medicines for Animals and Feed” (Protocol No. 125 of April 16, 2020).

Sample collection

Samples used in the study were obtained from wild free-living cervids, which were shot during the winter hunting seasons 2019-2023 in 15 different districts Moscow region (Figures 1-3). Samples were taken from the following animal species: from 144 moose (*Alces alces*); 19 roe deer (*Capreolus capreolus*); 15 animals without species information.

Total 78 nasal swabs and 371 tissue samples, including parts of the nasal septum, upper tracheal rings, lung, heart, liver, kidneys and pooled organ samples were tested.

Detection of viral nucleic acids

Nasal swabs and frozen organ samples from all animals were subject to DNA and RNA isolation. Total nucleic acids were extracted from 100 µL of the 10% suspension of tissue or swab sample using the RIBO-prep kit (AmpliSens, Russia).

Previously published PCR protocols were used to detect BVDV, BoHV-1 and other α-herpesviruses (Pchelnikov et al., 2023), BoHV-4 and BoHV-6 (Yatsentyuk et al., 2022), SARS-COV-2 (Krasnikova et al., 2022). For RNA of BCOV, RVA and

PIV-3 detection were used RT-PCR kits "BOVINE CORONAVIRUS-FACTOR", "ROTAVIRUS-FACTOR", and "PIV-3-FACTOR" (VetFaktor, Russia). PCR and RT-PCR for the nucleic acids of adenoviruses, HEV and BRSV were made with oligonucleotides and conditions shown in the Table 1.

Conventional PCR was carried on "Tercyk" Multi-block Thermocycler (DNA-technology, Russia) in a 25 µL reaction mixture containing 2.5× PCR-mix2 blue (AmpliSens), 10 mM of dNTPs, 0.6 µM of both forward and reverse primers. RT-PCR amplification mixture contains 5X One-Step RT-PCR Mastermix (Belbiolab, Russia), 0.6 µM forward and reverse primers, 0.3 µM fluorescent probe. Real-time PCR was carried on a RotorGene Q (Qiagen, Germany). Conventional PCR products from all samples were analyzed by electrophoresis in 1.8% agarose gel and visualized under ultra violet light. The selected PCR products were submitted for sequencing.

Sequencing of purified amplicons was carried out using the BrilliantDye V3.1 reagent kit on an Applied Biosystems 3100 Genetic Analyzer (Life Technologies, USA). The obtained nucleotide sequences were analyzed using the BLAST algorithm on the Internet search resource of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Maps were constructed using ArcMap10.8 program (Esri, Redlands, California, USA).

Table 1 - List of oligonucleotides for HEV, BRSV and Bovine adenovirus-3 PCR detection

Virus	Target region	Primer Sequence 5'-3'	Size (bp)	Tm	Reference
ADV	E2A	Forward: GAGATGGATGTGAACAGCGA Reverse: ACATTCTGATGCTGGTACTG	644	95°- 5 min, 45 cycles (95°- 20 c, 55°- 20 s, 72°- 40 s), 72°- 5 min	Zhu et al. (2011)
BRSV	gene N	Forward: GCAATGCTGCAGGACTAGGTATAAT Reverse: ACACTGTAATTGATGACCCCATTCT TaqMan probe: R6GACCAAGACTTGTATGATGCTGCCAAAGCABHQ	124	50°- 30 min 95°- 15 min, 10 cycles (95°- 10 s, 60°- 20 s, 72°- 10 s), 35 cycles (95°- 10 s, 55°- 20 s detection, 72°- 10 s), 72°- 5 min	Boxus et al. (2005)
HEV	ORF3	Forward: GGTGGTTTCTGGGGTGAC Reverse: CGAAGGGGTTGGTTGGATG TaqMan probe: Cy5GGGTTGATTCTCAGCCCTTCGCBHQ	73	50°- 30 min 95°- 15 min, 5 cycles (95°- 10 s, 60°- 20 s, 72°- 10 s), 40 cycles (95°- 10 s, 55°- 20 s detection, 72°- 10 s), 72°- 5 min	Jothikumar et al. (2006)

ADV: Bovine adenovirus-3; BRSV: bovine respiratory syncytial virus, HEV: Hepatititis E virus

RESULTS

DNA of herpesviruses BoHV-4, BoHV-6, CvHV-1, CvHV-2 and ElkHV-1 was not found by conventional PCR in all 178 animals. Also, all samples were negative for BCoV, SARS-COV-2, PIV-3, HEV and BRSV. The PCR results for RVA, BVDV, BoHV and Adenoviruses are shown in Table 2. The location of the shooting sites of positive animals is displayed on the map of the Moscow region (Figure 2)

Adenoviral DNA was founded in swabs and respiratory samples (parts of the nasal septum and trachea) of 4 moose, 1 roe deer and 1 animal without information about species. Animals were hunted in Yegoryevsk (N=1), Stupino (N=1) and Pavlovsky-Posad districts (N=4).

Analysis of the nucleotide sequences of the 644 bp amplicons, obtained by conventional PCR with primers to Bovine adenovirus 3, showed only 83.97% identity with E2A region of Bovine adenovirus 3. All sequences of 6 positive PCR samples from different cervids were identical. A partial DNA sequence of the virus named Roe deer adenovirus 1 from a sample of a roe deer hunted in 2022 has been deposited in the GenBank database (GenBank: ON936732.1).

Group A Rotavirus RNA was detected in pooled organ samples from 3 moose hunted in Stupino, Lukhovitsy and Rusa districts of Moscow region. BVDV RNA was founded in nasal swab of moose from Serpukhov district. Viral concentration in samples was low, the average Ct value during real-time PCR for BVDV was 28.33, for RVA -33.10.

Bovine herpesvirus DNA was detected in samples from 9 animals. It was found in the nasal swabs of 2 roe deer hunted in 2019 in Lukhovitsy district. In 2022-2023 hunting season BoHV DNA was detected in organ samples of 2 moose (in a lung sample of one moose, as well as in samples of the kidney and heart of another animal) from Stupino; in the nasal swab of 1 roe deer from Serpukhov; in the pooled organ samples of 2 roe deer from Lukhovitsy and 2 moose from Klin and Orekhovo-Zuevo districts. The average Ct value during real-time PCR was 26.8.

Analysis of the nucleotide sequences of the herpesviral gB gene fragment, obtained as a result of sequencing PCR products with primers common to α-herpesviruses, confirmed the presence of BoHV-5 DNA in a nasal swab sample from a roe deer and BoHV-1 DNA in two moose samples. Due to the low quality of the samples, it was not possible to identify the types of bovine herpesviruses in the remaining animal samples.

Table 2 - Results of PCR detection of group A Rotaviruses, bovine herpesviruses 1 and 5, bovine viral diarrhea virus, and adenoviruses with species and number of animals sampled in brackets

Positive PCR results / Animal species tested	`RVA (%)	ADV (%)	BVDV (%)	BoHV (%)
Moose (Alces alces) (N=144)	0	1 (5.26)	0	5 (26.31)
Roe deer (*Capreolus capreolus*) (N=19)	0	1 (6.67)	0	0
Deer without species information (N=15)	3 (1.68)	6 (3.37)	1 (0.56)	9 (5.05)
Total (N=178)	3 (1.68)	6 (3.37)	1 (0.56)	9 (5,05)

E.P.C.<0.05

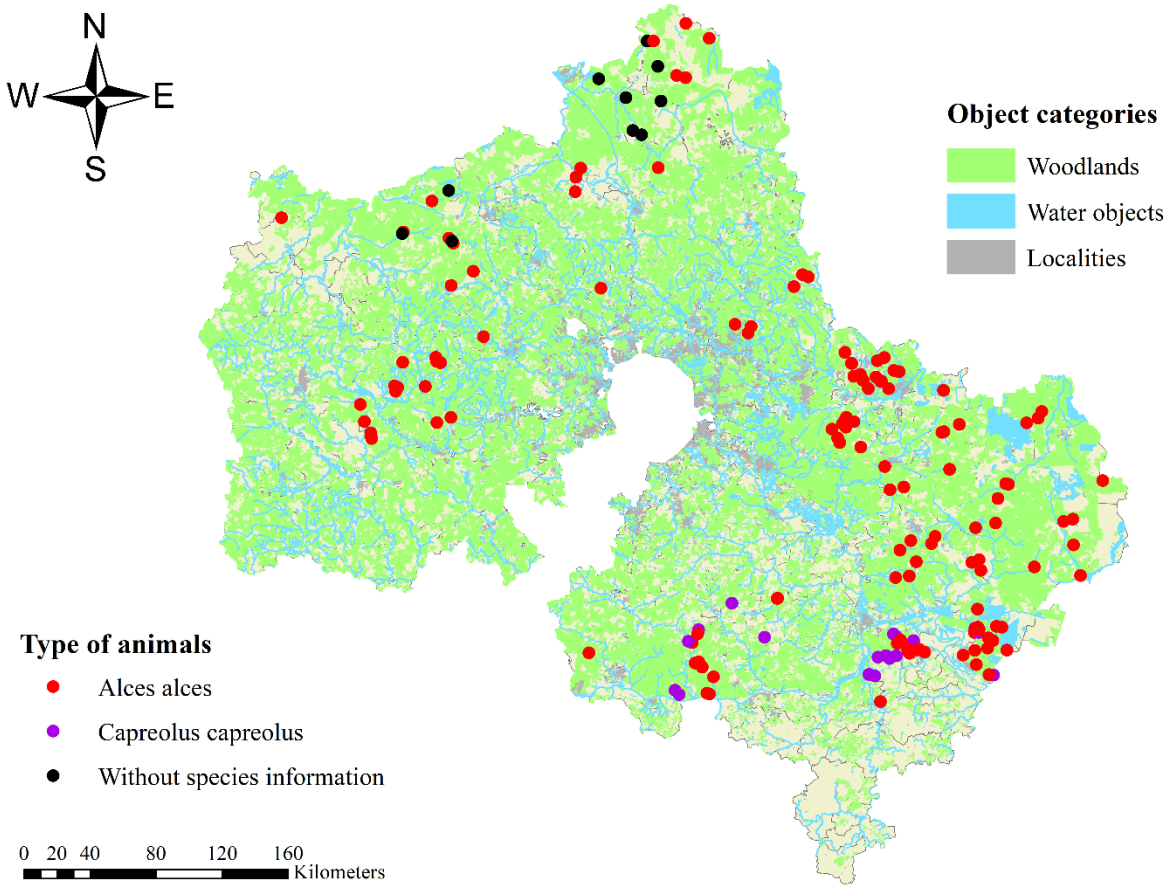

Figure 1- A map of Moscow region depicting sampling sites

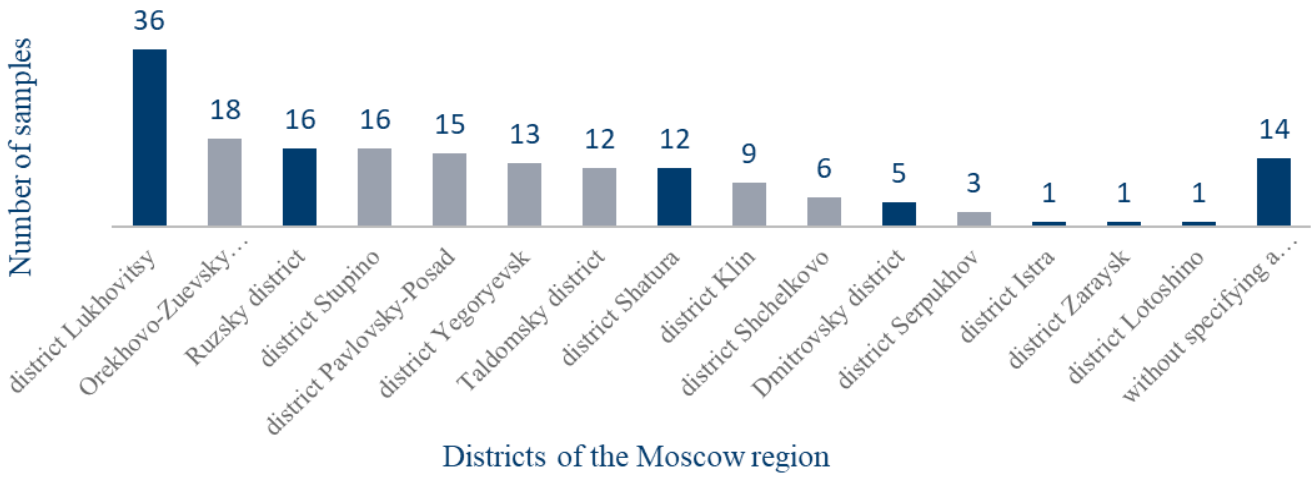

Figure 2 - Distribution of studied samples by Moscow region samples

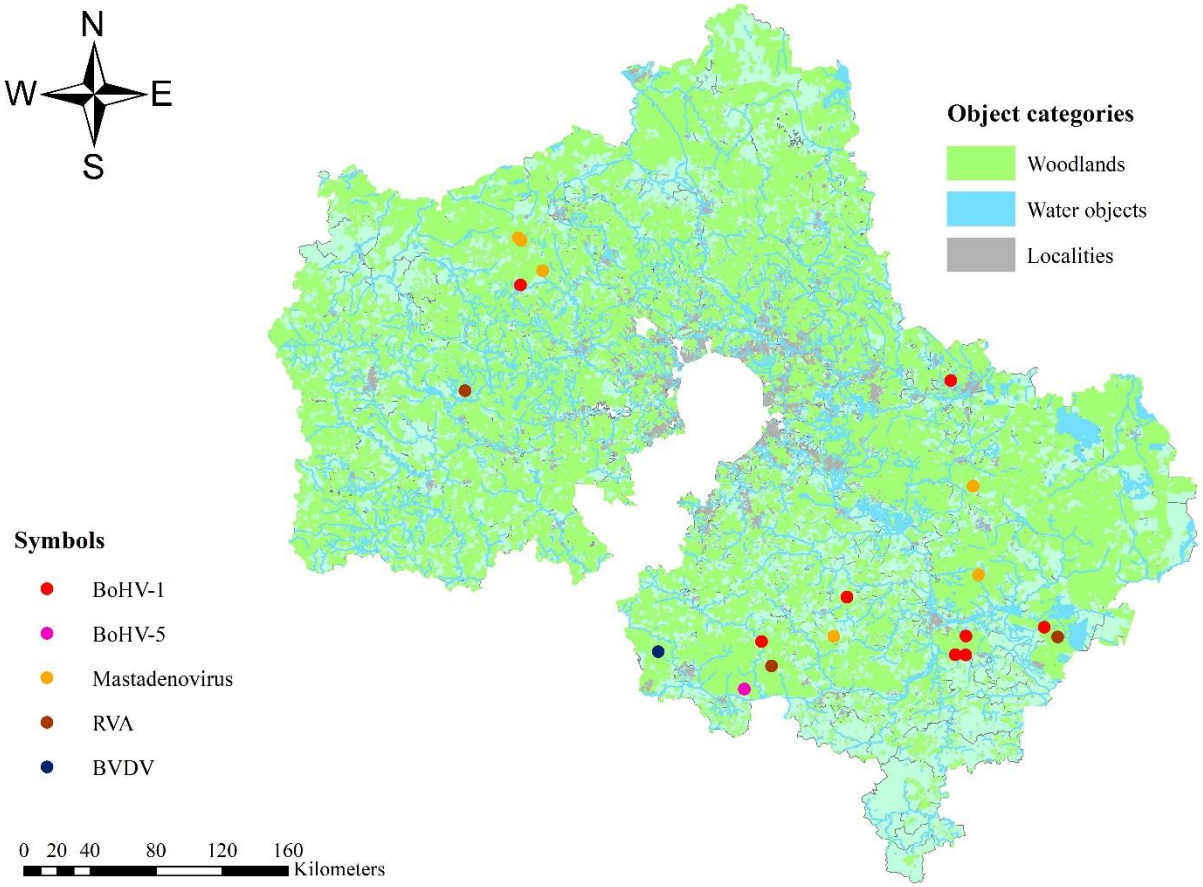

Figure 3 - The location of the shooting sites of positive animals on the map of the Moscow region

DISCUSSION

In the studies, was detected the DNA of bovine herpesviruses in various pathological material of 9 animals out of 178. The sampling was limited to districts of the Moscow region, and the sampling size for moose was significantly larger than the sampling for roe deer. In general, this data corresponds to the results of BoHV-1 DNA detection in European roe deer (Kálmán and Egyed, 2005). In a study of cervids in Hungary, BoHV-1 DNA fragments were found in 21.4% roe deer samples. More than 12.5% of samples from roe deer contained BoHV-4, but BoHV-5 DNA was not detected (Kálmán and Egyed, 2005). In the study no BoHV-4 DNA were detected, but in nasal swab of one roe deer sample was detected BoHV-5 DNA. These differences are likely due to different animal tissues tested.

The PCR method used to detecting DNA of α-herpesviruses in swabs and upper respiratory tract during the period of active replication of viral DNA, which this method is commonly used in equine viral tests (Pusterla and Leutenegger, 2015). For BoHV-1, the period of active shedding is 5-14 days after infection. During the latent period, when herpes viruses remain in the nerve cells, there are not detected in biological fluids and upper respiratory tract by PCR. In the study nerve tissues were not tested. The positive PCR result obtained in the study indicates not only the presence of herpesviruses, but also its activation in the animal. It can be assumed that the activation of BoHV-1 in wild cervids may be caused by climate change, as well as stressful situations.

The results of RVA studying correlate with the data of Shulyak et al., who demonstrated the presence of rotavirus RNA in moose in the Moscow region (Shulyak et al., 2020). Although intestinal tissues associated with rotavirus replication were not examined in this assay, viral RNA was detected in the pooled samples. This may indicate that the tissues were contaminated during sampling and necropsy. Previous genotyping of RVA strains revealed unique types in roe deer and fallow deer (Althof et al., 2023). Unfortunately, the low content of RVA RNA in the samples did not allow us to determine the genotype of the virus in moose.

The detection of BVDV RNA in a moose sample indicates the circulation of BVDV in the population of moose in the Moscow region. The question of whether wild cervids can be a source of BoHV-1 and BVDV for cattle remains controversial. Although animals on small farms can graze freely on summer pastures, contact between wild and domestic ungulates for viral transmission is limited. Additional studies of the genetic relationships of BoHV and BVDV isolates circulating in wild and domestic ungulates to determine if moose or roe deer can be infection reservoir are required. The

results obtained from identifying adenoviruses in moose and roe deer suggest the circulation of a new virus in cervids. Based on the results of phylogenetic analysis, it can be concluded that the detected adenovirus belongs to the genus Mastadenovirus. The detected virus probably affects the respiratory tract of animals, and most likely does not cause an acute disease.

The final classification of any new virus should be based on the most complete study of its biological properties and features of genome organization. A necessary tool for studying the genome is next-generation sequencing technology, which, if a sufficient amount of virus accumulates, allows one to decipher the structure of the genome in a short time. Unfortunately, in most cases, in vitro cultivation of wild animal adenoviruses and assessment of its biological characteristics is difficult due to the lack of appropriate cell cultures. In such cases, as noted in a review article on animal adenoviruses, one must rely on information obtained from deciphering individual fragments of DNA, which, despite its limitations, provides information about the diversity of adenoviruses (Harrach et al., 2019).

CONCLUSION

In conclusion, among the viral pathogens with zoonotic potential - hepatitis E virus and group A rotavirus, only rotavirus was detected in the studied animals. SARS-COV-2, which was detected in white-tailed deer, was not detected in cervids samples from Moscow region. However, to reduce the risk of transmission of viral pathogens, it is important to observe measures to prevent zoonotic diseases when interacting with animals, cutting carcasses, and consuming wild deer meat products. For the first time, a new mastadenovirus has been detected in different deer species, likely causing a mild respiratory disease. The work showed that viral infections do not pose a danger to the deer population of various species in the Moscow region. Further research is needed to study the biological characteristics of the new viral pathogen. Molecular studies indicate the circulation of BoHV-1, BoHV-5, BVDV in cervids in the Moscow region. Some authors point to the role of wild artiodactyls as a natural reservoir of viral pathogens of livestock diseases. This research work does not suggest a direct link between cervid infections and livestock diseases in the same region. The potential transmission routes of viruses between wild ruminants and livestock are still poorly understood. Additional molecular genetic studies are needed to confirm the circulation of the same strains among hunting animals and livestock.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

S. Yatsentyuk designed the study, M. Krasnikova writing the manuscript. K. Dolinskaya collecting samples and data. A. Pchelnikov analysis data and manuscript writing. All authors drafted and revised the manuscript as read, evaluation and approved the final manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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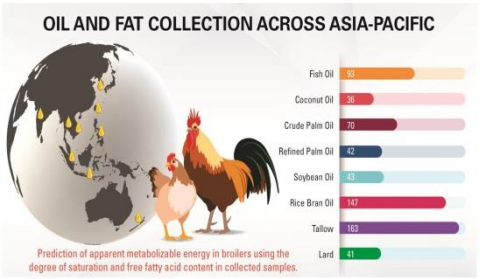

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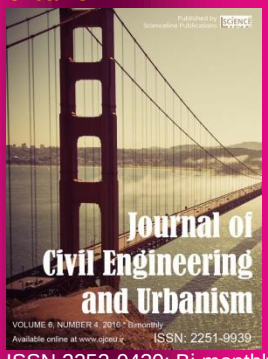

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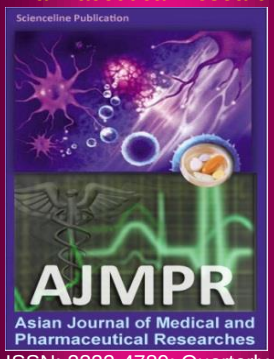

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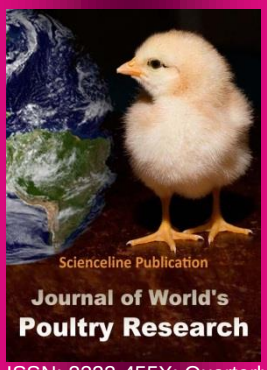

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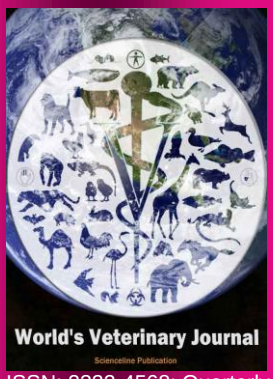

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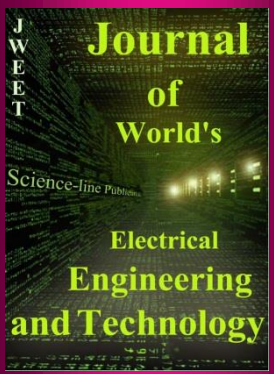

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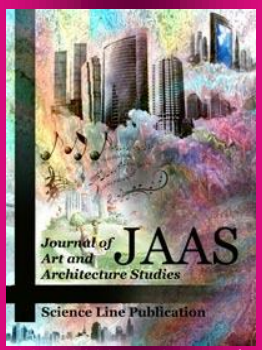

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