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Volume 13 (6); November 25, 2023

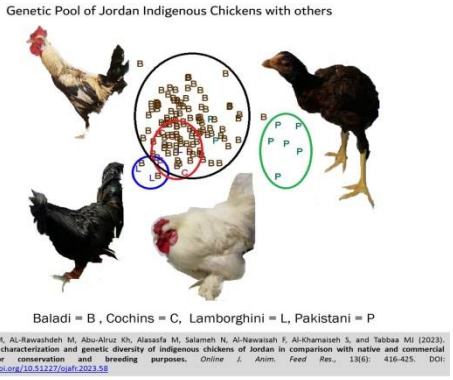
Research Paper

Phenotypic characterization and genetic diversity of indigenous chickens of Jordan in comparison with native and commercial breeds for conservation and breeding purposes

Al-Atiyat RM, AL-Rawashdeh M, Abu-Alruz Kh, Alasasfa M, Salameh N, Al-Nawaisah F, Al-Khamaiseh S, and Tabbaa MJ.

Online J. Anim. Feed Res., 13(6): 416-425, 2023; pii: S222877012300058-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.58



Abstract
Indigenous chickens of Jordan are facing extinction and need genetic conservation because they were unable to commercially compete in the poultry industry because of low genetic ability compared to commercial layers. The study aimed to investigate phenotypic and genetic diversity of village chickens in Jordan using discriminant analyses procedures to provide a basis for sustainable genetic conservation and utilization program to overcome any possible extinction. The sampled chicken population of 578 one-year old chickens (125 males and 433 females) was phenotypically characterized for 15 biometric and plumage traits from major cities of the three regions; Middle, North, and South. The traits variations within and between breeds were detected statistically by stepwise discriminant and canonical-discriminant of uni- and multivariate analyses. The results showed the sampled population as village chickens in Jordan is comprised of indigenous (Baladi) breed (85%) and few exotic and commercial breeds. The breeds were distinct and differentiated based on phenotypic traits indicating high genetic variability. The major phenotypic traits that showed significant power to differentiate breeds were comb type, body weight, comb size, earlobe color, wattle size, face color and breast size in males and comb type and size, body weight, face and breast size, leg color and wattle size in females. Recent and past crossings, admixture or migration from exotic and commercial breeds were noted. Moreover, low levels of phylogeographic structure were observed across the studied breeds. In conclusion, there is need to conserve the indigenous breed *in situ* and *in vivo* for its adaptive gene pool in coming days of persisted climate change and diseases threats.
Keywords: Breed conservation, Distance, Genetic diversity, Morphology, Native chickens.

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Research Paper

The process of yak milk fermentation by polycomponent starter culture

Usubalieva A, Musulmanova M, Saalieva A, Ozbekova Z, Aralbek kyzy A, and Deidiev A.

Online J. Anim. Feed Res., 13(6): 426-432, 2023; pii: S222877012300059-13

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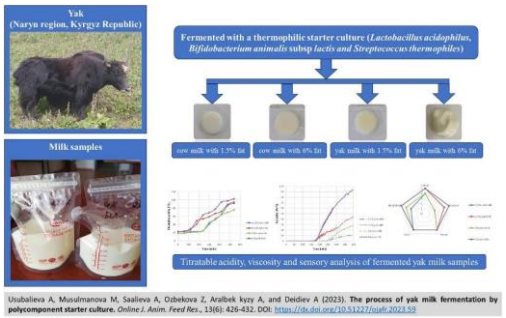

Abstract
The paper presents a comparative characteristic of the fermentation processes of yak and cow milk samples with a high fat content of 1.5% and 6% with a multicomponent starter culture, which includes *Lactobacillus acidophilus, Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus.* Acid formation in the process of milk fermentation under the influence of the starter microflora was assessed by the dynamics of changing in titratable (Ac) and active (pH) acidity over time. The course of the formation of the structure of the resulting clot was monitored on a rheometer, fixing the viscosity characteristics of the fermented milk clot in dynamics. It has been established that the increase in acidity occurs more intensively in yak milk in comparison with cow's milk with a corresponding acceleration of the formation of a fermented milk clot. In conclusion, the resulting clots were subjected to sensory analysis with the identification of the best sample, which was fermented yak milk with a fat mass fraction of 6%.
Keywords: Cow milk, Fermentation, Rheological properties, Starter culture, Yak milk.

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Research Paper

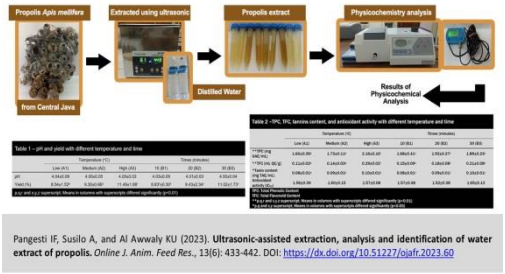
Ultrasonic-assisted extraction, analysis and identification of water extract of propolis

Pangesti IF, Susilo A, and Al Awwaly KU.

Online J. Anim. Feed Res., 13(6): 433-442, 2023; pii: S222877012300060-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.60

Abstract



Pangesti IF, Susilo A, and Al Awwaly KU (2023). **Ultrasonic-assisted extraction, analysis and identification of water extract of propolis.** *Online J. Anim. Feed Res.*, 13(6): 433-442. DOI: https://dx.doi.org/10.51227/ojafr.2023.60

Apis mellifera is one species of bee that produces propolis, a resin-based product. Propolis extraction using ultrasonic assistance is being widely studied. Using water as a solvent is a challenge to capture the bioactive components of propolis. This research aimed to determine the physicochemical quality resulting from the processing of propolis extract from Central Java by ultrasonics using water as a solvent at different temperatures and times. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. The study used nine treatments with three replications. The extraction time was carried out for 10, 20, and 30 minutes. The study used nine treatments with three replications. The results of the analysis showed that propolis extraction at different temperatures and times had a very significant effect ($P<0.01$) on the yield, total phenolic content (TPC), and total flavonoid content (TFC), with an average of 6.7–13.3%, 1.10–2.21 mg GAE/mL, and 0.07–0.32 mg QE/mL, respectively. Propolis extraction at different temperatures and times had no significant effect on tannin content, pH, and antioxidant activity. Regarding yield, TPC, TFC, and tannin content values, it was determined that extracting at high temperatures for 30 minutes produced the best results. High temperatures and long timespans are used for the best chance of collecting bioactive components.

Keywords: Bee products, Physicochemical, Processing, Propolis extract, Water solvent.

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Research Paper

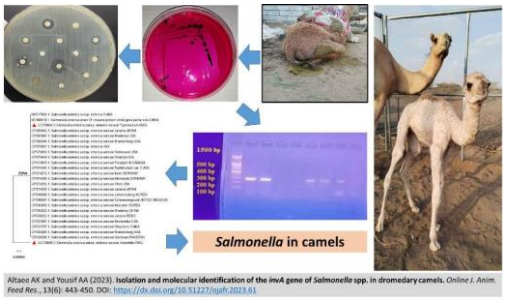
Isolation and molecular identification of the *invA* gene of *Salmonella* spp. in dromedary camels

Altaee AK and Yousif AA.

Online J. Anim. Feed Res., 13(6): 443-450, 2023; pii: S222877012300061-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.61

Abstract



Altaee AK and Yousif AA (2023). **Isolation and molecular identification of the *invA* gene of *Salmonella* spp. in dromedary camels.** *Online J. Anim. Feed Res.*, 13(6): 443-450. DOI: https://dx.doi.org/10.51227/ojafr.2023.61

This study was done to determine the percentage of *Salmonella* spp. in camels from three provinces (Karbala, Al-Najaf and AL-Muthana) in Iraq with different age and both sexes. Total of 250 fecal samples from 250 camels were collected. Diagnostic study depended upon the morphological and cultural properties of the isolates on some selective media like Xylose lysine deoxycholate (XLD) and *Salmonella Shigella* (SS) agars which were used in addition to different biochemical tests and molecular assay by PCR for detection of virulence gene invasion A (*invA*) with Phylogenetic study. The clinical signs appearing on animals infected with *Salmonella* were greenish diarrhea, loss of appetite with mild systemic reaction. Bacteriological and molecular tests revealed isolation of five *Salmonella* isolates with invA gene. Two of these isolates were sequenced. The results showed that the first strain *S. enterica subspecies typhimurium* (LC730846) converged with a group of global strains with one node, as it converged with the global strain that held the clade (MK017934.1 and MT460418.1). While the second local strain *S. enterica serovar enteritidis* (LC730849) appeared with a new node and it is not affiliated with any association with the world *S. enterica* strains. It is concluded that the presence of *Salmonella* spp. in camels needs monitoring in order to minimize the risks of infection exposing human beings.

Keywords: Camels, Fecal samples, *invA* gene, PCR, *Salmonella*.

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Research Paper

Productivity of the Tsigai sheep breed under different feeding regimens

Kitaeva A, Mamedova V, Bezaltychna O, Slyusarenko I, and Novichkova A.

Online J. Anim. Feed Res., 13(6): 451-459, 2023; pii: S222877012300062-13
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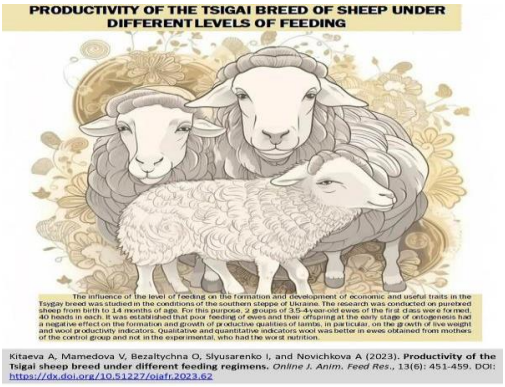

Abstract
In the present study, the influence of levels of feeding on the formation and development of economic and commercial traits of the Tsigai breed was studied in the conditions of the southern steppe of Ukraine. The research was conducted on purebred sheep from birth to 14 months of age. For this purpose, 2 groups of 3.5-4 years old ewes of the first class were selected with 40 heads in each class. It was established that poor feeding of ewes (experimental diet and below standard nutritional levels) and their offspring at the early stage of ontogenesis had a negative effect on the formation and growth of productive qualities of lambs, means of live weight and wool productivity indicators. Qualitative and quantitative indicators of wool were better in ewes obtained from mothers of the control group (who received a balanced diet in accordance with the standard of feeding). Advantage in length of wool at 12 months age was 29.3%, shearing of unwashed wool (26.7%), washed (26.5%), strength of wool at 4 months of age (10.5%), and in the 14th month aged was 5%. The improvement in housing and nutrition conditions in the control group proved that the counts were better and this had a very positive effect on the productivity of the sheep. In conclusion, full-fledged feeding of ewes of the Tsigai breed ensured good development of offspring at all stages of ontogenesis and contributed to the birth of healthy, viable lambs that are capable of high productivity. Any decline in nutrients of Tsigai sheep breed (from standards of commercial formula) can cause considerable deficiency in productivity of animals.
Keywords: Feeding, Live weight, Local breeds, Productivity, Wool quality.

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Research Paper

Morphology and reproductivity profiling of male Senduro goats based on age differences

Ducha N, Lisdiana L, Trimulyono G, Izzatunnisa F, Yusof NNM, Samsulrizal N, Dasiman R, Fudhaili A, Pramesti GRA, and Rahayu JD.

Online J. Anim. Feed Res., 13(6): 460-466, 2023; pii: S222877012300063-13
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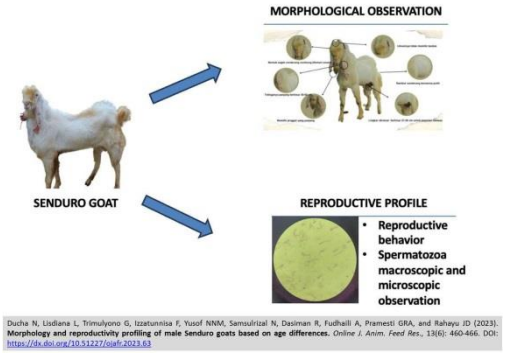

Abstract
Senduro goats, a local breed of meat and dairy goats from Indonesia, are recognized for their significance in improving goat breeding and preserving valuable genetic resources. However, limited information exists regarding the reproductive physiology of Senduro goats, which poses challenges to the development of breeding programs and the preservation of genetic resources. This study aimed to investigate the morphological and reproductive profiles of male Senduro goats at different ages, focusing on morphological characteristics, mating behavior, and sperm quality. Morphological characteristics are assessed through body length measurements, while mating behavior serves as an indicator of reproductive behavior. Macroscopic evaluations of sperm quality include assessments of color, viscosity, pH, and volume, while microscopic examinations encompass motility (mass and individual), viability, and spermatozoa membrane integrity. The results showed morphological similarities between juvenile and adult samples, with their testicular size being the only significant difference. Based on macroscopic and microscopic examinations, no significant differences were found between groups. From the results it was concluded that there were no distinct differences in morphological characteristics, mating behavior, and sperm quality between male Senduro goats in the juvenile and adult stages.
Keywords: Biometric assessment, Mating behavior, Morphology profiles, Semen quality, Senduro goat.

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PHENOTYPIC CHARACTERIZATION AND GENETIC DIVERSITY OF INDIGENOUS CHICKENS OF JORDAN IN COMPARISON WITH NATIVE AND COMMERCIAL BREEDS FOR CONSERVATION AND BREEDING PURPOSES

Raed M. AL-ATIYAT[1], Mustafa AL-RAWASHDEH[2], Khaled ABU-ALRUZ[3], Muawya ALASASFA[4], Naser SALAMEH[4], Firas AL-NAWAISAH[2], Sami AL-KHAMAISEH[2], and Mohammad J. TABBAA[5]

[1]*Genetics and Biotechnology, Department of Animal Production, Mutah University, Jordan*
[2]*Department of Animal Production, Mutah University, Mutah 60710, Karak, Jordan*
[3]*Department of Nutrition and Food Processing, Faculty of Agriculture, Mutah University, Karak, 61710, Jordan*
[4]*Department of Plant Production, Faculty of Agriculture, Mutah University, Karak, 61710, Jordan*
[5]*Department of Animal Production, School of Agriculture, The University of Jordan, Amman, Jordan*

Email: ratiyat@mutah.edu.jo

Supporting Information

RESEARCH ARTICLE
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ABSTRACT: Indigenous chickens of Jordan are facing extinction and need genetic conservation because they were unable to commercially compete in the poultry industry because of low genetic ability compared to commercial layers. The study aimed to investigate phenotypic and genetic diversity of village chickens in Jordan using discriminant analyses procedures to provide a basis for sustainable genetic conservation and utilization program to overcome any possible extinction. The sampled chicken population of 578 one-year old chickens (125 males and 433 females) was phenotypically characterized for 15 biometric and plumage traits from major cities of the three regions; Middle, North, and South. The traits variations within and between breeds were detected statistically by stepwise discriminant and canonical-discriminant of uni- and multivariate analyses. The results showed the sampled population as village chickens in Jordan is comprised of indigenous (Baladi) breed (85%) and few exotic and commercial breeds. The breeds were distinct and differentiated based on phenotypic traits indicating high genetic variability. The major phenotypic traits that showed significant power to differentiate breeds were comb type, body weight, comb size, earlobe color, wattle size, face color and breast size in males and comb type and size, body weight, face and breast size, leg color and wattle size in females. Recent and past crossings, admixture or migration from exotic and commercial breeds were noted. Moreover, low levels of phylogeographic structure were observed across the studied breeds. In conclusion, there is a need to conserve the indigenous breed *in situ* and *in vivo* for its adaptive gene pool in the coming days of persisted climate change and disease threats.

Keywords: Breed conservation, Distance, Genetic diversity, Morphology, Native chickens.

INTRODUCTION

The sustainability of biodiversity and genetic resources is considered the first step towards food security in each country. Thus, many countries nowadays apply the convention on biological diversity (CBD) as main regulation and law to govern species biodiversity (Chandra and Idrisova, 2011). Regarding domestic animals and livestock, the CBD of each country develop a strategic action plan for their genetic diversity conservation and genetic resources utilization. The genetic resources are representing ability of genetic makeup or livestock breed to produce and reproduce in specific conditions. Nowadays, commercial breeds have been genetically improved for high production in intensive system. On the other hand, indigenous or native (Baladi –Arabic name–) breeds are surviving better in low-input and village production systems. In fact, farmers and villagers are replacing indigenous breeds with commercial strains and/or their crossbreds. This practice is considered main threat of indigenous chickens' biodiversity in developing countries resulting in losing genetic resources as well as chicken extinction. The Food and Agriculture Organization of the United Nations (FAO, 2004) recommended, in such a situation of extinction, to apply appropriate management and conservation strategies. In general, livestock extinction is globally concerned for conserving genetic resources of future need and utilization (FAO, 2008). Nevertheless, utilization of biodiversity is the main aim of countries' national plan which include collecting and disseminating information and applying practical practices to conserve biodiversity. Particularly, the practical application of genetic conservation is contested by farmer breeding practices, global warming, exotic breeds/trains, and social-economic issues (Hoffmann, 2022).

Jordan, since the 1970s, has developed commercial poultry farms by importing high producing exotic layers and

Citation: Al-Atiyat RM, AL-Rawashdeh M, Abu-Alruz Kh, Alasasfa M, Salameh N, Al-Nawaisah F, Al-Khamaiseh S, and Tabbaa MJ (2023). Phenotypic characterization and genetic diversity of indigenous chickens of Jordan in comparison with native and commercial breeds for conservation and breeding purposes. *Online J. Anim. Feed Res.*, 13(6): 416-425. DOI: https://dx.doi.org/10.51227/ojafr.2023.58

broilers to meet the increasing demand of eggs and meat. Major developments, in the poultry industry, have occurred in 2000s in which large numbers of high-producing strains of layers are released into market and reared in villages and suburban areas without governmental regulation prevent unplanned crossbreeding with the indigenous chickens. Indigenous chickens (*Gallus gallus*) of Jordan are domesticated chicken reared in villages and backyards with low input requirements and conditions. Their phenotypes are features for numerous variations in body shape and size, feather and leg color, comb type and size, slow growth rates and small egg size (Abdelqader et al. 2007; Al-Atiyat, 2009). The latter two features were the reasons why some farmers crossed them with exotic breeds to benefit from heterosis effects or hybrid vigor towards more egg and meat production (Ahmed et al., 2020). Jordanian farmers are in general practicing crossbreeding of exotic commercial layers and ornamental breeds and ancient breeds (Al-Atiyat, 2009). It is worthy to mention, the most common threat to the indigenous chicken's diversity of the word is crossbreeding (Leroy et al, 2016). Consequently, avoiding crossbreeding practices by farmers is important step to conserve indigenous chicken genetic resources and their genetic variations. Furthermore, conserving genetic variation is priority for both current and future utilization indigenous chicken that most tolerable breed of persisted global warming in Jordan. Additionally, Jordan indigenous chicken has better interest for avoiding extinction afterward the last decade outbreak of avian flu (poultry influenza) which associated with extensive culling to control the epidemic (Dunn et al., 2019).

Worthwhile, phenotypic description of indigenous chicken is characterizing and documenting all genes that contribute to phenotypic and plumage traits. This is considered genetic characterizations which is a prerequisite to sustainable conservation plan (FAO, 2013). So that the plan is comprised of evaluation phenotypic traits, breeding history, genetic diversity level within and between populations which is measured by statistical analysis such as multivariate discriminant procedure (González Ariza et al., 2021). The objective of the study was to assess the genetic diversity of Jordan indigenous chickens for detecting the conservation possibility and future perspective under current climate situation.

MATERIALS AND METHODS

Chicken population

First, this study is interested in the indigenous or Baladi chickens which were found in rural and urban areas of Jordan. Sampled population was village chickens which found in these areas, and it was comprised of various breeds; indigenous, ancient exotic and commercial breeds. We sampled mainly the indigenous individuals and some predefined exotic and commercial individuals as a reference groups. The sampled indigenous chicken population were classified and named after each sampled governorate from three major regions of Jordan: North, Middle and South regions. The other sampled breeds were predefined exotic breeds of Cochins, Fayoumi, Lamborghini and Pakistani. In addition to commercial breeds of Hy-Line White and ISA Brown chickens. The Cochin chicken is a breed of large feather-legged chickens come from China (Larkina et al., 2021). Fayoumi chicken breed is robust breed in a harsh environment and native to Egypt (Dessie et al., 2011). Lamborghini chicken is all black in appearance known as Ayam Cemani chicken comes from Java (Indonesia) and nicknamed the Lamborghini of all chicken breeds because of their price, rarity, and prestige (Dharmayanthi et al., 2017). Pakistani chicken breed is thought to have evolved from cockfighting chickens and being native to Pakistan and thus called Pakistani in Jordan (Abdelqader et al., 2007). The Hy-Line White chickens and ISA Brown chicken are modern strains of commercial layers producing eggs. ISA Browns are brown-feathered and brown egg layers, and Hy-lines chickens are white-feathered and white egg layers.

Ethical Regulations and considerations

In this research, handling chickens was practiced with the permission of the appropriate regulations and guidelines of the Ethics Committee of Mutah University (No.: AGR/1/15/2018).

Data recording and statistical analysis

The plumage and biometric data were documented following pictorial guidelines of chickens phenotypic characterization (FAO, 2008). The traits were body weight and color, comb color, type and comb size, beak color, face color and size, wattle color and size, ear lob, eye and leg color, and breast color and size. The survey was executed to ensure random sampling. The chickens of one-year old were randomly selected, weighted and phenotypic traits were recorded. The total population size was 558 individuals: 125 males and 433 females.

The statistical analyses were based on the SAS program version 9.2 (SAS, 2010). The first analysis was phenotypic clustering model (PCM). Second, simple discriminant analysis was performed to calculate probability of an individual chicken into predefined group. Then stepwise discriminant was also used to define traits of better discriminating power. Last, canonical -discriminant analysis of uni- and multi-variate analysis- was performed to generate canonical variables (CAN). The CANs were counted for pairing each breed with other breeds into one genetic group and cluster of potential population or breed. Finally, the Mahalanobis distances were estimated of the covariance matrix (Winaya et al., 2023). Consequently, the SAS TREE procedure was operated to build a dendrogram using the statistical method of unweighted pair's group. Finally, the PROC CLUSTER procedure was accomplished utilizing distances data to form the clusters.

Citation: Al-Atiyat RM, AL-Rawashdeh M, Abu-Alruz Kh, Alasasfa M, Salameh N, Al-Nawaisah F, Al-Khamaiseh S, and Tabbaa MJ (2023). Phenotypic characterization and genetic diversity of indigenous chickens of Jordan in comparison with native and commercial breeds for conservation and breeding purposes. *Online J. Anim. Feed Res*., 13(6): 416-425. DOI: https://dx.doi.org/10.51227/ojafr.2023.58

RESULTS

Phenotypic traits and discriminant power

Table 1 shows the phenotypic traits and sampling location details. The results showed the sampled population as village chickens in Jordan is comprised of indigenous (Baladi) breed (85%) and many exotic and commercial breeds; Cochins (2.5%), Fayoumi (1.5%), Hy-Line (3.5%) , ISA-brown (1%), Lamborghini (2%), Pakistani (4%). The contribution of samples per governorate varied from the highest in Krak (21.5%) to lowest by Tafilh governorate (5.5%).

Diversity and differentiation analyses

The Biometric and plumage variable showed a wide range of differentiation ability (Table 2). Five traits only (body weight, wattle size, earlobe color, face color, comb size) were significantly able to differentiate individuals of the breeds in males. They were body weight, wattle size, earlobe color, face color and comb size which abled to significantly separated the breeds' males. In females, the variables were comb size, body weight, face size, comb type, leg color, breast size, and wattle size that significantly discriminated them between pairwise females' breeds on average. Body weight of males was the most powerful discriminant variable, while comb size of was the most powerful discriminant variable.

The results of discriminant procedure showed Pakistani male chickens were highly differentiated ($P<0.0001$) from others (Table 3). The indigenous male breed was also significantly differentiated from Pakistani breed with the longest distance value. On the other side, the Mahalanobis distances of the female breeds were significantly differentiated ($P<0.001$). The longest distance was between Baladi female breed and the Hi-Line female breed. The lowest nonsignificant distance was between Baladi and Fayoumi breeds. In general, the longest significant distance was noted between Baladi and each of commercial chickens (Hy-Line White -White Leghorns- and ISA Brown laying hens -Rhode Island Red chicken-) breeds, reflecting the long genetic distance between them (Table 3).

Table 4 shows Eigenvalue, variance proportion, and canonical correlation variables. Function Canonical value number one (CAN1) is qualified to the differences among the males of the breed. The high percentage of Eigenvalue variation (63.6% and 75.2% for male and female, respectively) and thus total variation in the grouping of discriminant function 1 in this study is related to the differences among males and females the breeds in the studied traits and evidence of high genetic variation (73.2% for male and 70.9% for female). In details, the variation proportion of males (70%) of function 1 was higher than other functions. Additionally, CAN1 is higher than the other functions' values. Likewise results of variation proportion and the canonical correlation of function were noted.

Table 1 - Frequency of Jordan indigenous chicken breeds, their sampling location of both males and females

Breed	No.	Percent (%)	No.	Percent (%)	Total No.	Overall Percent (%)
	Females		Males		Females and Males	
Baladi	364	84	108	86	472	85
Cochins	4	1	5	4	9	2.5
Fayoumi	14	3	-	0	14	1.5
Hy-Line	30	7	-	0	30	3.5
ISA brown	7	2	-	0	7	1
Lamborghini	5	1	4	3	9	2
Pakistani	9	2	8	6	17	4
Total	433	100	125	100	558	100
Governorate						
Ajlun	30	7	11	9	41	8
Aman	20	5	5	3	25	4
Aqba	35	8	17	14	52	11
Blqa	45	10	10	8	55	9
Jrsh	17	4	5	4	22	4
Krak	90	21	27	22	117	21.5
Maan	79	18	18	15	97	16.5
Mdba	59	14	15	12	74	13
Mfrq	35	8	10	8	45	8
Tfilh	23	5	7	6	30	5.5
Total	433	100	125	100	558	100

Citation: Al-Atiyat RM, AL-Rawashdeh M, Abu-Alruz Kh, Alasasfa M, Salameh N, Al-Nawaisah F, Al-Khamaiseh S, and Tabbaa MJ (2023). Phenotypic characterization and genetic diversity of indigenous chickens of Jordan in comparison with native and commercial breeds for conservation and breeding purposes. *Online J. Anim. Feed Res*., 13(6): 416-425. DOI: https://dx.doi.org/10.51227/ojafr.2023.58

Table 2 - Summary of stepwise selection of traits

Entered	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Squared Canonical Correlation	Pr > ASCC
Male							
Body weight	0.1302	6.04	0.0001	0.8698	0.0007	0.0434	0.0007
Wattle size	0.0907	3.99	0.0095	0.7909	<.0001	0.0707	0.0001
Earlobe color	0.0657	2.79	0.0437	0.739	<.0001	0.0899	<.0001
Face color	0.0723	3.07	0.0307	0.6855	<.0001	0.1085	<.0001
Comb size	0.0634	2.64	0.0529	0.6421	<.0001	0.1293	<.0001
Female							
Comb size	0.3567	39.37	<.0001	0.6433	<.0001	0.0595	<.0001
Body weight	0.1029	8.12	<.0001	0.5771	<.0001	0.0746	<.0001
Face size	0.0737	5.62	<.0001	0.5346	<.0001	0.0853	<.0001
Comb type	0.0736	5.6	<.0001	0.4952	<.0001	0.096	<.0001
Leg color	0.0486	3.6	0.0017	0.4711	<.0001	0.1032	<.0001
Breast size	0.036	2.62	0.0168	0.4542	<.0001	0.1087	<.0001
Wattle size	0.0233	1.67	0.1268	0.4436	<.0001	0.112	<.0001

Table 3 - Mahalanobis distance and Prob > Mahalanobis distance between Males and Females of the chicken breeds

Male breed					Cochins	Lamborghini	Pakistani
Baladi					2.83^{NS}	4.93^{NS}	9.51***
Cochins						5.286^{NS}	13.92***
Lamborghini							19.16***
Female breed	**Baladi**	**Cochins**	**Fayoumi**	**Hy-Line**	**ISA brown**	**Lamborghini**	**Pakistani**
Baladi		2.13^{NS}	1.53^{NS}	9.52***	9.70***	1.33^{NS}	7.94***
Cochins			1.59^{NS}	14.52***	13.39**	1.41^{NS}	8.38^{NS}
Fayoumi				14.92***	10.47***	1.67^{NS}	8.85***
Hy-Line					26.45***	13.16***	26.86***
ISA brown						9.78*	16.37***
Lamborghini							10.73**

* :P<0.05, **: P<0.01 ***: P<0.001 , NS: not significant.

Table 4 - Eigenvalue, variation proportion, and canonical correlation of each function for males and females

Canonical	Eigenvalue	Variation proportion	Canonical correlation	Pr > F
Males				
1	0.636	0.732	0.624	0.001
2	0.146	0.168	0.357	0.620
3	0.087	0.100	0.283	0.729
Females				
1	0.752	0.709	0.655	<.0001
2	0.154	0.145	0.365	<.0001
3	0.105	0.099	0.308	0.144
4	0.032	0.030	0.177	0.978
5	0.015	0.015	0.123	0.998
6	0.003	0.003	0.054	1.000

The genetic contribution of chicken individuals' breed into the overall genetic pools of breeds is shown in Table 5. It is noted that 60.19% of Baladi males shared pure genetic pool of Baladi, and 22.22% of its genetic pool hared with Cochins. Fewer proportions were noted between Baladi males and males of Lamborghini and Pakistani breeds. However, good proportion of males' assignment (40%) as an error rate indicated that misassignment of crossbreds or non-Baladi males as Baladi breed. On the other hand, almost exotic breeds were assigned with high proportion to their own breed (80, 75 and 75% for Cochins, Lamborghini and Pakistani breeds, respectively. The results also showed that most females Baladi (27.75%) were assigned to unique genetic pool of Baladi chicken

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followed by Lamborging and Fayoumi with fair proportion; 19.78 and 16.48%, respectively. However, near 72% of Baladi females were misclassified as pure Baladi. For instance, 21.43% of Fayoumi and 3.33 % of Hy-Line females were assigned as Baladi females. On the other hand, 50% of Cochine and Fayoumi breeds were predefined Baladi chicken were sharing 50% of their genetic pools as phenotype with Baladi females. The results are indicated by error rate (Table 5). The results might indicate a shared genetic pool between Baladi breeds and the exotic studied breeds or crossbreds.

Based on previous results of CAN's function 1 in which CAN1 exhibited the major variations for males and females. The variations in the CAN1 for both males and females was related to the following phenotypic variables, the body weight and color, comb color, peak color and face color. Thus, they allowed for a clear distinction between male breeds (Table 6). For more details in females, the Can values showed high correlated coefficients between combinations of the plumage traits of chicken breeds indicating that comb type is most discriminant variable.

Furthermore, the results showed significant coefficient values for CAN1 of male breed in which Pakistani breed had higher value for CAN1. Similarly, it was noted higher value of female Pakistani in CAN1 (Table 6). Better illustration of the results is seen as plotted in Figure 1 in which male and female breeds differentiated along CAN1 axis and Can2 axis. Thus, the canonical variables were successful in differentiating breeds' sexes from each other. In details, individuals of the breeds distributed in either separated group or intermixed group indicating how phenotypically and genetically close. In other words, the results illustrate how individuals of both sexes from breeds were related based on individual principal component analysis. For example, the first genetic group of the male plumage in Figure 1 is represented by black circle grouped where most samples are Baladi males except few individuals of Colachins, Lamborghini and Pakinstani. Both exotic breeds of Lamborghini and Pakinstani chickens are grouped in separated circle each. Pakistani males formed in one separated and distinct group (green circle). On the other hand, this principal component analysis shows a clustering of females as presented individuals of different breeds plotted with others providing evidence of clear separation, intermixing, or crossbred in Baladi breed. However, major samples of Baladi are not crossbred individuals and thus they were clustered together from all sampled regions of Jordan. In fact, more obvious results were noticed for the female individuals indicating that the individuals were grouped from same breed representing their closeness in sharing sample variables (Figure 1). However, some Baladi individuals

Table 5 - Membership proportion (%) between studied chicken breeds

Male					Female						
Breed	**Baladi**	**Cochins**	**Lamborghini**	**Pakistani**	**Breed**	**Baladi**	**Cochins**	**Fayoumi**	**Hy-Line**	**ISA brown**	**Lamborghini**	**Pakistani**
Baladi	60.19	22.22	10.19	7.41	Baladi	27.75	10.71	16.48	10.71	5.49	19.78	9.07
Cochins	20	80	0	0	Cochins	0	50	0	0	0	50	0
Lamborghini	25	0	75	0	Fayoumi	21.43	7.14	50	0	0	14.29	7.14
Pakistani	25	0	0	75	Hy-Line	3.33	0	0	96.67	0	0	0
					ISA brown	0	0	0	0	85.71	14.29	0
Priors	0.25	0.25	0.25	0.25	Lamborghini	0	20	0	0	0	80	0
Rate	0.40	0.2	0.25	0.25	Pakistani	0	22.22	0	0	0	0	77.78
					Priors	0.14	0.14	0.14	0.14	0.14	0.14	0.14
					Rate	0.72	0.50	0.50	0.03	0.14	0.20	0.22

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of both sexes did not reflect this relationship; they were clustered with other breeds or located in a place far away.

To highlight how the Baladi can be utilized for its purity, genetic resources, and conservation, separated clusters were reconstructed for dendrogram tree of different branching level for males and females (Table 7). For males, the first branch included Baladi chickens breed formed in a separate cluster or genetic group or gene pool with Cochins (Figure 2). The second branch formed from males of Lamborghini breed. The last branch had Pakistani males far away from other branches or breeds' genetic pools. The dendrogram of females (Figure 3) shows a cluster of Baladi in separated branch. The second cluster was a sub-group that includes Cochins and Lamborghini and Fayoumi chickens. This group of sub-clustered with Cochins and Fayoumi breeds forming the first major cluster as one group / gene pool close to Baladi chickens. The rest breeds were far separated from those breeds in a third cluster of branches. Pakistani breed was grouped with the ISA brown instead Baladi indicating an intermediate position Hy-Lin and ISA brown. The female dendrogram branching was like the males' except that three breeds (Fayoumi, Hy-Lin and ISA brown) were missed in males' dendrogram.

Table 6 - Total-sample standardized canonical coefficients, and total variations explained by each canonical variable (CAN).

Variable	Male				Female			
	CAN1	CAN2	CAN3	CAN4	CAN1	CAN2	CAN3	CAN4
Body weight	0.00108	0.00006	-0.00037	-0.00035	0.00062	-0.00168	0.00192	0.00061
Body color	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	-0.00001
Comb color	0.00036	0.00095	-0.00043	-0.00017	0.00000	0.00004	-0.00006	0.00002
Comb type	-0.14447	0.33245	-0.08804	0.17721	-0.15034	0.28590	-0.04539	0.16189
Comb size	-0.00475	0.04714	-0.02647	-0.00885	-0.08415	-0.00398	0.01888	-0.01554
Peak color	0.00038	0.00065	-0.00126	-0.00064	0.00004	0.00002	0.00007	0.00000
Face color	0.00240	-0.00010	-0.00047	0.00012	-0.00004	-0.00007	-0.00011	0.00009
Face size	0.00051	0.00930	0.05111	0.03171	0.02153	0.07010	0.00581	-0.00871
Wattle color	0.00070	-0.00005	-0.00089	0.00181	-0.00003	0.00070	0.00063	0.00037
Wattle size	-0.04848	-0.04855	-0.02255	0.06530	0.02708	0.00945	-0.06279	0.04872
Earlobe color	0.00054	0.00041	0.00077	-0.00025	0.00000	0.00000	-0.00002	0.00001
Eye color	-0.00157	0.00090	0.00017	0.00021	-0.00035	0.00016	-0.00007	0.00070
Breast color	0.00008	-0.00002	0.00000	0.00008	0.00000	0.00001	-0.00001	0.00007
Breast size	0.02873	-0.00047	0.00107	-0.00331	0.01378	0.02955	0.00217	-0.09914
Leg color	-0.00003	0.00032	0.00047	0.00181	0.00096	0.00185	0.00295	-0.00017

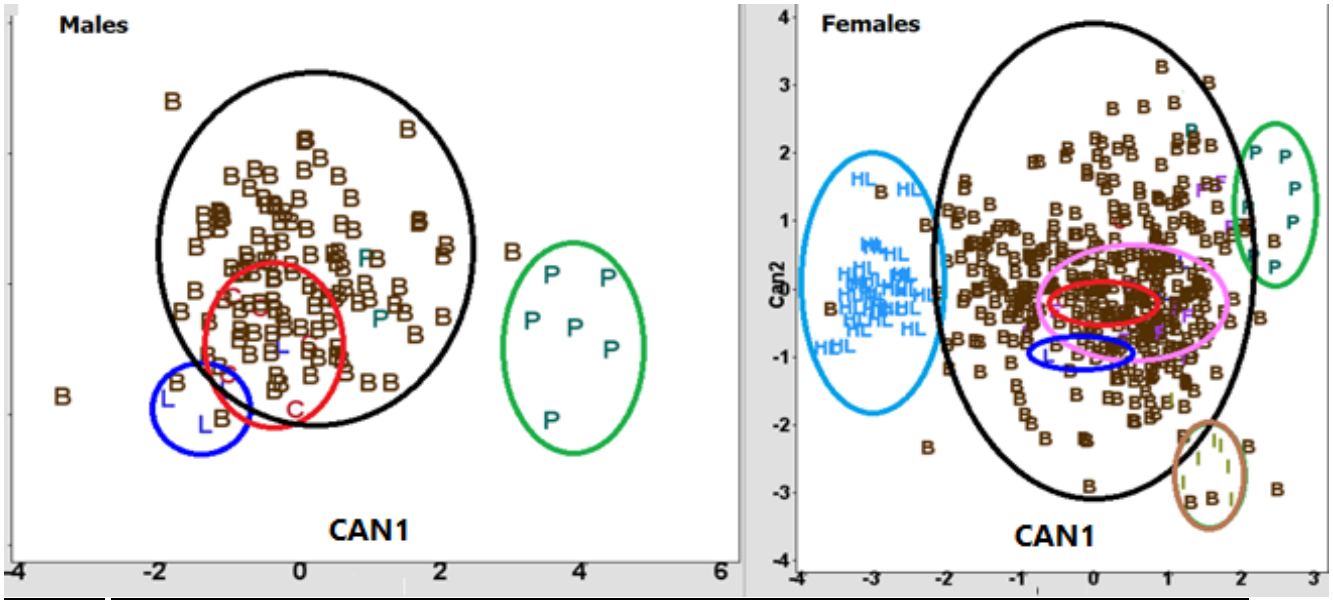

Figure 1- Discriminating both sexes of the breeds based on canonical variables. (Breeds: Baladi = B; Cochins = C; Fayoumi = F; Hy-Line = HL; ISA brown= I; Lamborghini = L; Pakistani = P).

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Table 7 - Canonical variables (CAN) values of each male and female breeds

Male breed	CAN1	CAN2	CAN3	Female breed	CAN1	CAN2	CAN3	CAN4
Baladi	-0.144	0.1301	-0.018	Baladi	0.1257	0.0096	-0.042	0.0663
Cochins	-0.564	-0.945	1.204	Cochins	0.5958	0.4087	-0.64	-0.755
Lamborghini	-1.21	-1.605	-0.901	Fayoumi	0.7465	-0.033	-0.69	-0.719
Pakistani	2.9001	-0.363	-0.054	Hy-Line	-2.933	0.0096	0.292	-0.173
				ISA brown	1.4743	-2.467	1.2305	-0.29
				Lamborghini	0.4605	-0.328	-0.657	-0.179
				Pakistani	1.8625	1.5506	1.5019	-0.328

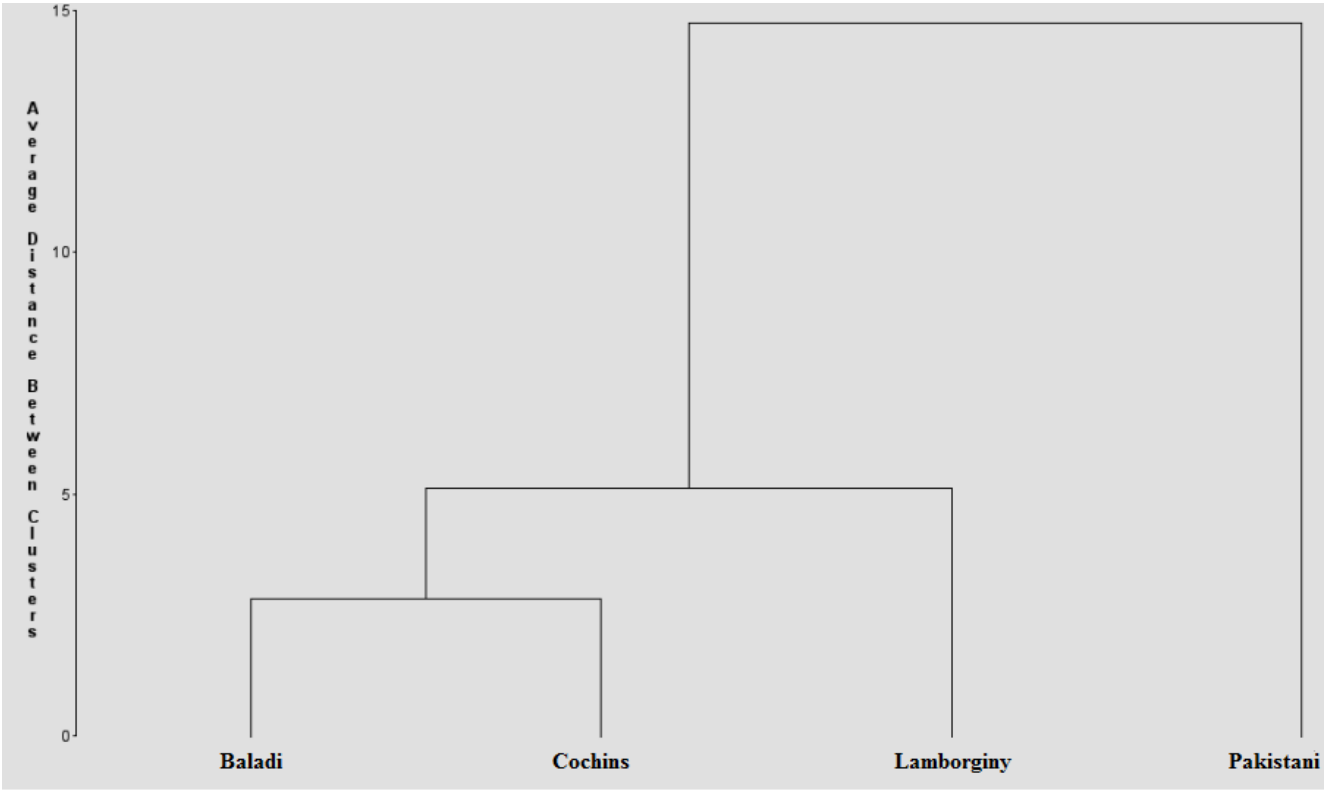

Figure 2 - The constructed dendrogram of males

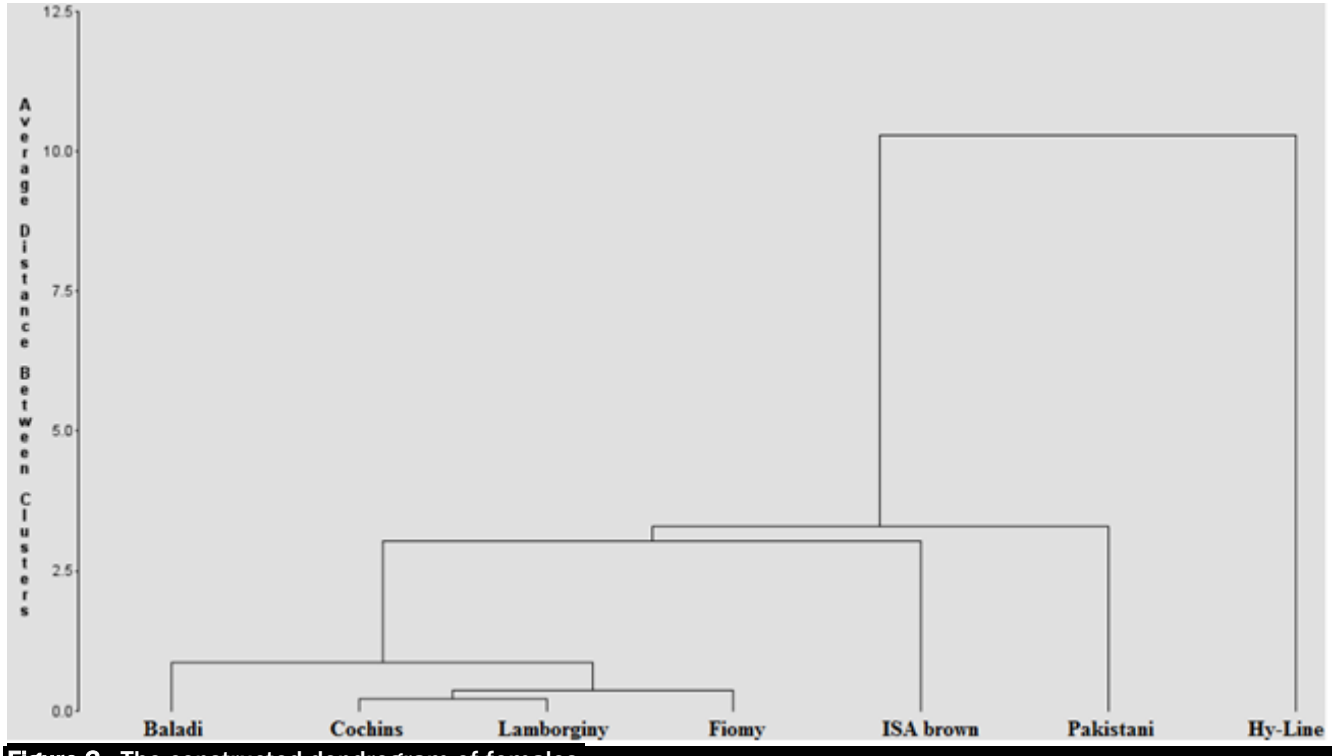

Figure 3 - The constructed dendrogram of females

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DISCUSSION

The indigenous chickens of Jordan are well adapted to the local climatic conditions of dominant heat stress and drought, scarce feed, with better resistance to diseases and stresses. Their phenotypic traits were developed because of natural and artificial selection throughout past ages. Considering their domestication and dispersal from literature, they were developed in different parts of the world from different old breeds and formed the present look like chicken phenotype. In fact, the phenotype and plumage traits of the chicken are like old and indigenous chickens of worldwide indigenous chickens. Furthermore, they were reared along with breeds come from countries as close as Egyptian (Fayoumi breed) and as far as Pakistan (Pakistani breed) (Abdelqader et al., 2007).

The results showed great variation in plumage and morphometric traits which might be a result of mainly selection and geographical proximate. Similar findings were reported for worldwide indigenous breeds of many countries (Al-Atiyat, 2009; Daikwo et al., 2011; Al-Atiyat et al., 2017). For example, in Jordan the average live weight, of Baladi chickens of Al-Karak Governorate, were 1201 and 1681 grams for females and males of one-year old (Al-Atiyat et al., 2023). In Algeria, the body weight of males was about 1.4 kg and that of females was about 1.1 kg (Dahloum et al., 2016). In Ethiopia, the body weight of males was 1.630 kg and females 1.370 kg for village chickens. In Saudi Arabia, the body weight of males was 1.50 kg and females 1.3 kg (Abudabos et al., 2017). It was found that the chickens of different geographical regions were distinct based on phenotype traits. The Jordan chicken clearly stated that breeds within the region was due to the geographical proximity along with long natural selection. The discriminant traits that separated the chickens from other studied breeds were comb type, body weight, comb size, earlobe color, wattle size, face color and breast size in males and plumage variables that significantly discriminated between pairwise breeds' comparisons were comb type and size, body weight, face and breast size, leg color and wattle size in females. Similar plumage variables were reported by Halima et al. (2007), Dana et al. (2010) and Adekoya et al. (2013). Furthermore, the canonical discriminant analysis explained the total co-variation between plumage traits of the chicken breeds. In particular, the multiple correspondence analyses showed the variation was accounted for by the CAN2 and CAN3, and thus the Canonical discriminant analysis was proved for successful identifying variation of phenotypic traits between breeds. It agrees with similar studies of worldwide chicken breeds (Rosário et al., 2008; González Ariza et al., 2022; Muluneh et al., 2023). Finally, based on phenotypic traits, the Mahalanobis distances show an expected differentiation for males and females breeds.

The large distances were observed for clearly distinct breeds; mainly the Pakistani, Hy-line and Issa Brown from other ones. It is worth mentioning that Baladi chicken was closer to the ancient exotic breeds and commercial breeds found in Jordan and reared long with for long time ago. Nevertheless, the result might explain why Baladi individuals were located within groups of other breeds. This demonstrates that the significant ($P<0.0001$) studied phenotype traits of the Baladi were in similar with others and able to discriminate them away or close. It was found that most males of exotic breeds were correctly assigned (100%) into each of own breed. However, the results showed less proportion (75-80%) for exotic females. The results prove that full description of phenotypic traits of Jordan chickens help with finding guideline of distinct traits for breed assignment and predefinition procedure for genetic conservation and breeding programs. In agreement, Larkina et al. (2021) proposed a phenotypic clustering model for breeding programs for local, commercial, and fancy breeds.

The history of Jordan chicken domestication can be inferred the uncovered origin and development. There is diversity across regions; Central (Amman, Madaba, Zarqa) areas show more diversity than the two populations from the South of the country and North. There is evidence of crossbreds or exotic breeds of chickens which were sometimes hard to assign as Baladi or otherwise. It is common that village chicken population consisted of the indigenous, crosses and exotic chickens. In general, indigenous chickens are better for their adaptability, hatchability and have reproduction performance than exotic and crossbreed chickens (Khan, 2008; Dzungwe et al., 2022). However, there is a need for practical research to improve the implementation of long-term crossbreeding programs in developing countries (Leroy et al., 2016) including Jordan. Summing up, clearly identified breeds and genetic diversity may be attributed to route of dispersion and arrival of domestic chicken in the country. It is worth remembering that the Central (Amman, Madaba, Zarqa) area of the country includes the capital Amman where there is a major sale yard market of chicken. Amman and Maddaba might receive chicken from different geographic areas across time. In addition, most commercial farms of chickens – mainly layers– are found near both regions.

CONCLUSION

The phenotypic and plumage variations between breeds were detected within Baladi breed. The breeds had distinct differentiation reflecting the existence of high genetic variability between studied breeds based on phenotypic traits. The traits showed significant ability to differentiate breeds were body weight, comb type and comb size, face

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color and breast size and wattle size in males and females. In addition to earlobe color for males and leg color females each only. Past and recent crossing and migration of the exotic and commercial breeds was notified. However, canonical discriminant analysis was capable to assess genetic differentiation of Baladi Jordan chicken breeds. On the other hand, this study presented further support for the origin of Jordan chickens as well as for the importance conserving. They still have a unique genetic pool with shared genes with other studied breeds which can be clearly reported by low phylogeographic structure across the studied breeds. In conclusion, there is always a need to conserve the breed in situ and in vivo for better knowing of its origin and utilization of the genetic resources for better tolerance of climate stresses and diseases. It is also recommended further studies based on the D-loop chicken mitochondrial DNA for determining purity and origin, and SNP chip studies for detecting adaptive genes and selection signatures.

DECLARATIONS

Corresponding author
Correspondence and requests for materials should be addressed to Dr. Raed M. AL-ATIYAT; E-mail: ratiyat@mutah.edu.jo

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

Authors' contribution
R.M. Al-Atiyat contributed to research concept, data collection and analyses and the write up of the manuscript. M. AL-Rawashdeh, Kh. Abu-Alruz, and M. Alasasfa contributed to technical and logistic support. N. Salameh, F. Nawiseh, S. Al-Khamaiseh and MJ Tabbaa contributed to experimental design, data collection and execution. RMA, SKH and MJT contributed to data analysis and writing final drafted manuscript. All authors have read and approved the final manuscript.

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Consent to publish
The authors agree to the publication of this manuscript.

Competing interests
The authors declare no competing interest.

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THE PROCESS OF YAK MILK FERMENTATION BY POLYCOMPONENT STARTER CULTURE

Aigul USUBALIEVA[1], Mukarama MUSULMANOVA[2], Altynai SAALIEVA[2], Zhyldyzai OZBEKOVA[1], Anara ARALBEK kyzy[1], and Anarseit DEIDIEV[1]

[1] *Kyrgyz-Turkish Manas University, 56, av. Aytmatov, Bishkek, 720044, Kyrgyz Republic*

[2] *I. Razzakov Kyrgyz State Technical University, 66, av. Aytmatov, Bishkek, 720044, Kyrgyz Republic*

Email: ausubalieva@manas.edu.kg

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ABSTRACT: The paper presents a comparative characteristic of the fermentation processes of yak and cow milk samples with a high fat content of 1.5% and 6% with a multicomponent starter culture, which includes *Lactobacillus acidophilus, Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus.* Acid formation in the process of milk fermentation under the influence of the starter microflora was assessed by the dynamics of changing in titratable (Ac) and active (pH) acidity over time. The course of the formation of the structure of the resulting clot was monitored on a rheometer, fixing the viscosity characteristics of the fermented milk clot in dynamics. It has been established that the increase in acidity occurs more intensively in yak milk in comparison with cow's milk with a corresponding acceleration of the formation of a fermented milk clot. In conclusion, the resulting clots were subjected to sensory analysis with the identification of the best sample, which was fermented yak milk with a fat mass fraction of 6%.

Keywords: Cow milk, Fermentation, Rheological properties, Starter culture, Yak milk.

INTRODUCTION

Milk is the most valuable food product and makes a significant contribution to the human diet (Pronko et al., 2020; Duguma, 2022). However, some countries of the world, such as Mongolia, Nepal, and China, have relied on milk from other animals like yak for the production of dairy products. Yak milk is unique dairy product in terms of organoleptic, physico-chemical, and technological properties, as well as nutritional value (Agyare and Liang, 2021; Singh et al., 2023). There are many factors that are attributed to these variations, including the type of animal, its health indicators, rearing conditions, feeding characteristics, climatic conditions, season, inter alia. In particular, studies of the composition of milk depending on the season revealed differences in lactose content, fractional composition of protein, fat and other components (Elemanova, 2022; Li et al., 2011). It should also be noted that the milk of different animal species has different biological potential. Studies have shown that yak milk is more nutritious than cow's milk (Elemanova, 2022, Saalieva and Usubalieva, 2020). Yak milk is distinguished by a higher content of fat - 5.5-7.2%, protein - 4.9-5.3%, milk sugar - 4.5-5.0% and minerals - 0.8-0.9% (Nikkhah, 2011), in contrast to the cow, where the corresponding figures were: 3.8%, 3.3%, 4.8% and 0.7% (Smirnova et al., 2020). The range of nutrient content varies depending on various factors: genotype, age and diet of the animal (Nikkhah, 2011; Saalieva and Usubalieva, 2020; Smirnova et al., 2020; Elemanova, 2022). In these study, milk from yak selected at Arpa pasture in the Naryn region of the Kyrgyz Republic contained protein, fat, and lactose in proportions of 4.8%; 4.95% and 5.1%, respectively (Aralbek kyzy et al., 2022).

The chemical composition of milk affects the technological process of obtaining dairy products (Elemanova, 2022). Therefore, the study of the influence of the chemical composition of alternative milk on its technological properties is of considerable scientific and practical interest for the production of various products. The range of dairy products produced from yak milk according to national recipes inherited from time immemorial is diverse, but their production has so far been adapted only for home conditions in rural areas. On an industrial scale, the processing of yak milk in the Kyrgyz Republic is difficult, since there is no developed regulatory documentation and scientifically based technology for dairy products from yak milk, which is of particular relevance in light of the predicted increase in the number of yaks in different regions of Kyrgyzstan (Saalieva and Usubalieva, 2020).

Among the variety of dairy products, fermented milk or fermented milk (diet) drinks are of particular interest from a physiological functionality point of view. For the preparation of these are used - pure cultures of lactobacilli or their combinations with other microorganisms (yeast, propionic acid bacteria) (Savaiano and Hutkins, 2021; Kaur et al., 2022).

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Most lactobacilli are now classified as probiotics, which in recent years have been key components of functional foods, of which dairy accounts for more than 65 percent (Taye et al., 2021; Fesseha et al., 2021).

The choice of starter cultures is influenced by the type of product and, accordingly, the technology for preparing fermented milk products. The study of the process of fermentation of milk, in particular yak milk, using various types of starter cultures, is the scientific basis for the technology of developing new fermented milk products. In this connection, the purpose of these studies was to study the acid-forming ability of a consortium of microorganisms (*Lactobacillus acidophilus, Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus*) in yak milk, as well as the rheological properties of the fermented milk clot formed during fermentation.

MATERIALS AND METHODS

Milk samples

Yak milk was obtained directly from high mountain pastures (Arpa) in the Naryn region of the Kyrgyz Republic. For the study, the average milk batch was taken, which was collected from 5 yaks, which had similar calving periods. After milking, the milk was filtered, poured in sterile plastic bags and transported to the laboratory in a special thermal bag (8±1^{0}C) for storing perishable products. The object of comparison was pasteurized cow's milk with a mass fraction of fat of 1.5% and 6%, obtained from a local supermarket (Bishkek, Kyrgyz Republic).

Starter cultures

A multistrain starter (Lyofast SAB 430A, Italy) containing pure cultures of lacto- and bifidobacteria (*Lactobacillus acidophilus, Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus*) was used as a starter culture. This starter culture is mainly used in the production of probiotic fermented milk products with a soft and delicate texture of medium viscosity. The optimal temperature for the growth of microorganisms is in the range of 37-45°C. The standard mode of operation of the starter culture starts at a temperature of 43°C, pH=4.5±0.15, the fermentation time is on average 6-7 hours.

Physical measurements

Determination of titratable and active acidity was carried out by standard methods (AOAC, 2005). Studies of the rheological properties of fermented milk samples were carried out on a Rheometer MCR-302 device (Anton Paar, Graz, Austria) with a cylindrical geometry (CC27-SN26341) in the time base mode. The choice of this instrument for measuring non-Newtonian fluids, which include fermented milk drinks, is due to the fact that the rheometer allows to analyze such media without disturbing their structures (Smanalieva et al., 2021). The control of the gel formation process and the control of this process make it possible to obtain a clot with the necessary structural and mechanical properties. The temperature was chosen in accordance with the recommended one (Elemanova et al., 2022), optimal for the growth of starter cultures (43 °C).

Sample preparation

Raw yak milk was separated on a “Neptune” laboratory separator with subsequent normalization according to the mass fraction of fat 1.5% and 6.0%. Normalized milk was pasteurized at a temperature of 90-95 °C with a holding time of 5-8 minutes and cooled to the fermentation temperature (43-45 °C). The starter culture was added into the prepared milk with thorough mixing at the rate of 1 UC/100 liters of milk. Then stirring was continued for 10 minutes and the acid-forming ability of lactobacilli in yak milk was studied. In a similar way, comparison samples were prepared for the study - cow's milk with a mass fraction of fat of 1.5% and 6.0%. The same samples (fermented cow's and yak's milk) were used to study the process of structure formation.

Sensory analysis

Sensory tests were done to evaluate fermented yak and cow milk samples. Descriptive tests were conducted using descriptors adopted from Drake (2007). Total of ten trained panellists evaluated sensory properties: colour, texture, odour, taste, mouthfeel, and overall acceptance of the fermented yak and cow milk samples. All panellists were trained under the “Sensory Analysis” study program.

RESULTS AND DISCUSSION

In this study, the biochemical properties and starter microflora activity of yak milk and the formation of the associated structure of the fermented milk clot were studied in order to establish technological parameters for the production fermented milk products from yak.

Patterns of acid formation during the fermentation of yak milk by lactobacilli

The main biochemical process that occurs during the production of most fermented milk products is lactose fermentation, the end product of which is lactic acid. Lactic acid determines the sensory properties of the product, namely

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texture, taste, and smell. The activity of acid formation of lactobacilli depends on a number of factors, including the composition of the nutrient medium in which they are located. Comparative studies were carried out on the acid-forming activity of lactobacilli in starter cultures added to cow's and yak's milk of different fat content. After adding the starter, the milk samples were placed in a thermostat with a temperature favorable for the starter cultures (43 °C). The parameters, determined every 60 minutes, were titratable (Ac) and active (pH) acidity (Figures 1 and 2).

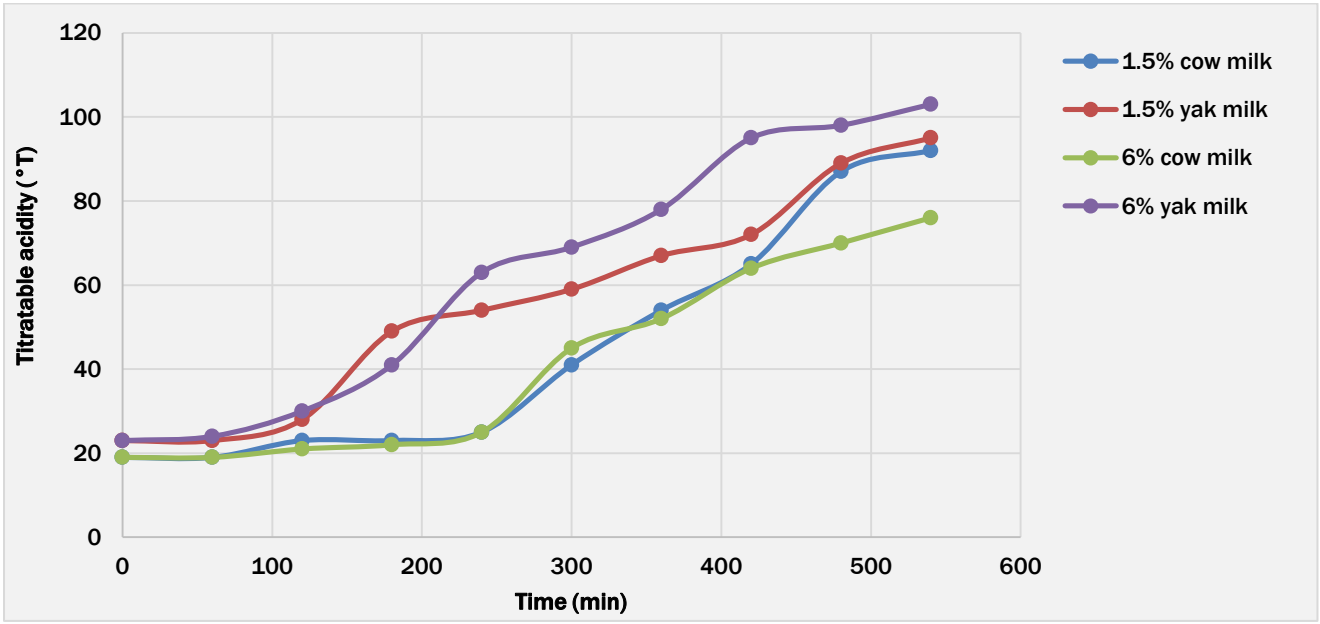

Figure 1 - Dynamics of changes in titratable acidity in yak and cow milk samples, fermented with a thermophilicstarter culture (*Lactobacillus acidophilus, Bifidobacterium animalis subsp Lactis and Streptococcus thermophilus*)

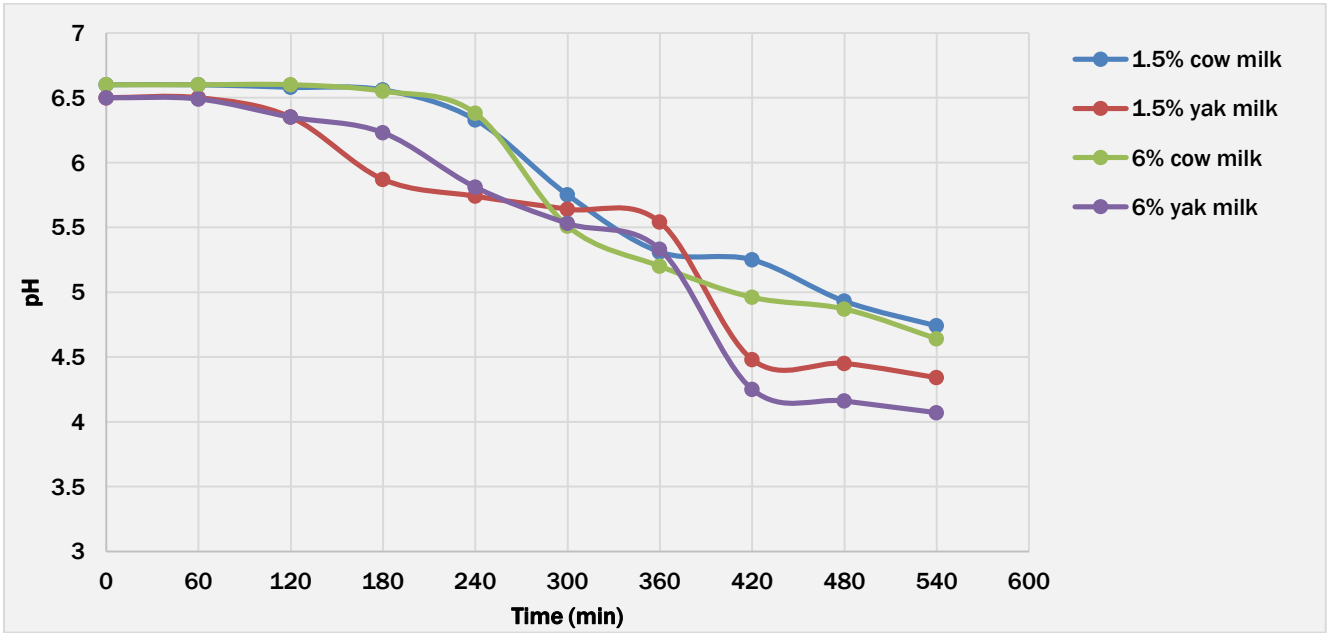

Figure 2- Dynamics of changes in active acidity (pH) in yak and cow milk samples, fermented with a thermophilic starter culture (*Lactobacillus acidophilus, Bifidobacterium animalis subsp Lactis and Streptococcus thermophilus*)

Figure 1 demonstrates the differences in the rate of passage of biochemical processes, namely lactic acid fermentation, in yak and cow milk. These differences are especially noticeable starting from the second hour of observations, when there is a rather sharp increase in titratable acidity in yak milk samples. At the 180 minute of fermentation, the difference between the acidity of yak and cow milk reaches almost 30 °T for 1.5% milk and about 20 °T for 6% milk. A similar sharp increase in titratable acidity for cow milk begins only after 4 hours from the moment the starter is added and until the end of fermentation, the rate of acid formation slowly decreases for 6% milk, reaching an acidity of 75 °T. For 1.5% cow milk, after almost the same fermentation process as 6% milk, at 420 minutes there is a second noticeable jump in the level of acidity, which is still difficult to explain reliably. Perhaps this is due to the species composition of the starter microflora, one of the components which is bifidobacteria. It is known that these microorganisms develop slowly in milk, because they are obligate anaerobes, and also do not show caseinolytic activity.

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These anaerobes can absorb casein only after partial hydrolysis (Funk and Irkitova, 2016). It can be assumed that at the first stages of fermentation, bifidobacteria do not participate in the fermentation of lactose for the reason indicated above. As the lactic acid fermentation develops, lactobacilli, capable of breaking down casein, lead to the formation of poly- and oligopeptides from it, stimulating the growth of bifidobacteria, which are now able to ferment lactose. In this regard, we can observe a second noticeable acceleration of acid formation, starting from the 420 minute of the process.

In this context, yak milk demonstrates similar dynamics in the development of lactic acid fermentation. After a noticeable increase in the level of acidity, there is a slight slowdown, starting from the 3 hour for 1.5% milk and from the 4 hour for 6% milk, and a second jump in acidity from 75 ºTh to 90 ºTh for 1.5% - milk from 7 to 8 hours of the fermentation process, and for 6% milk - from 80 ºT (6 hours) to 95 ºT (7 hours). At the same time, the minimum desired acidity of the final product, equal to 75 ºT, is achieved after 540 minutes for 6% cow's milk and after 340 minutes for 6% yak milk. This indicates an intensification of the technological process of processing the latter into fermented products. This is also evidenced by the moment of the beginning of the formation of a fermented milk clot, which occurs when the acidity reaches about 35 ºT. Such acidity is reached at the 140 minute for yak milk and at the 270 minute for cow milk.

A similar picture can be observed in Figure 2, which shows the dynamics of changes in active acidity in the fermented samples of the studied milk and the object of comparison.

The obtained data indicate that the studied yak milk is a favorable nutrient medium for the development of starter microflora with a reduction in the duration of the technological process for the production of fermented milk products by more than 3 hours.

Patterns of acid coagulation of yak milk proteins in the process of its fermentation by a polycomponent starter culture

The lactic acid formed during the fermentation of lactose under the action of lactobacilli causes coagulation of casein, which leads to gel formation of milk, i.e. the colloidal system from a freely dispersed state (structureless) changes into a coherently dispersed state (structured - gel) (Bylund, 1995). And this process is the most important in the development of fermented milk products, and the consistency, thixotropy, and structural integrity of the finished product depend on this process. The formation of the structure of a fermented milk clot during the fermentation of milk samples with a multicomponent starter culture was observed by changing the viscosity on a Rheometer MCR-302 device. The results of the analysis are presented in Figure 3.

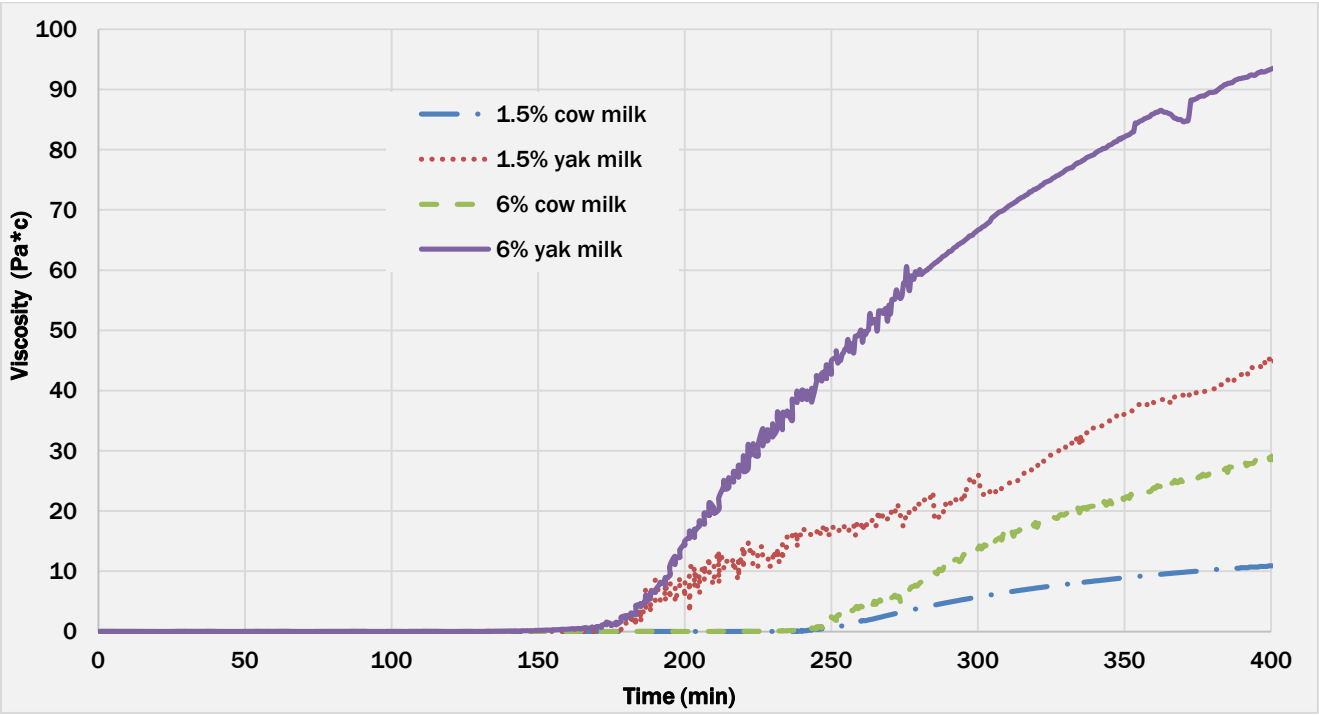

Figure 3 - Viscosity change in the process of structure formation during fermentation of samples of yak and cow milk with polycomponent starter.

Figure 3 shows the duration of individual stages of acid coagulation of yak and cow milk proteins differs markedly. In particular, the induction period of the structure formation process in yak milk lasts 180 and 200 min for 6% and 1.5% samples, respectively, while for cow milk this period lasts 250 min. This more than an hour difference is primarily due to the composition of milk, because all other experimental conditions are equal for the two studied types of milk. the amount of casein and the size of casein micelles determine the rate of acid coagulation of proteins and the strength of the resulting clots (Bylund, 1995). The influence of the composition of milk, namely the content of protein and lactose, on

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the process of gel formation is also confirmed by the data of other researchers (Elemanova et al., 2022; Yang et al., 2018).

Yak milk is characterized by a high protein content (4.9-5.3%) (Nikkhah, 2011), which leads to a reduction in the induction period of structure formation under the influence of lactic acid. Yak milk also contributes to a significant increase in the viscosity of the formed clot at the 400 minute in comparison with cow milk fermentation - about 3 times for 6% milk and 4 times for 1.5% milk. The obtained data indicate the intensification of the technological process for the production of fermented milkdrinks from yak milk with the formation of clots with improved rheological characteristics.

Sensory analysis of fermented yak and cow milk

The results of the analysis carried out by a group of tasters consisting of 10 people are presented in Figure 4. All samples had a pleasant sour-milk taste and smell. Samples of fermented yak milk had a slight specific taste. The consistency of the clots differed significantly according to t-tests (paired-sample tests) with a 95% confidence interval with SPSS software (SPSS Inc., Chicago, IL, USA). When fermented, yak milk gives a well-formed clot with a creamy, viscous consistency, while cow milk has a more viscous clot, characteristic of acidophilic drinks. The best performance was noted for a sample obtained from 6% yak milk.

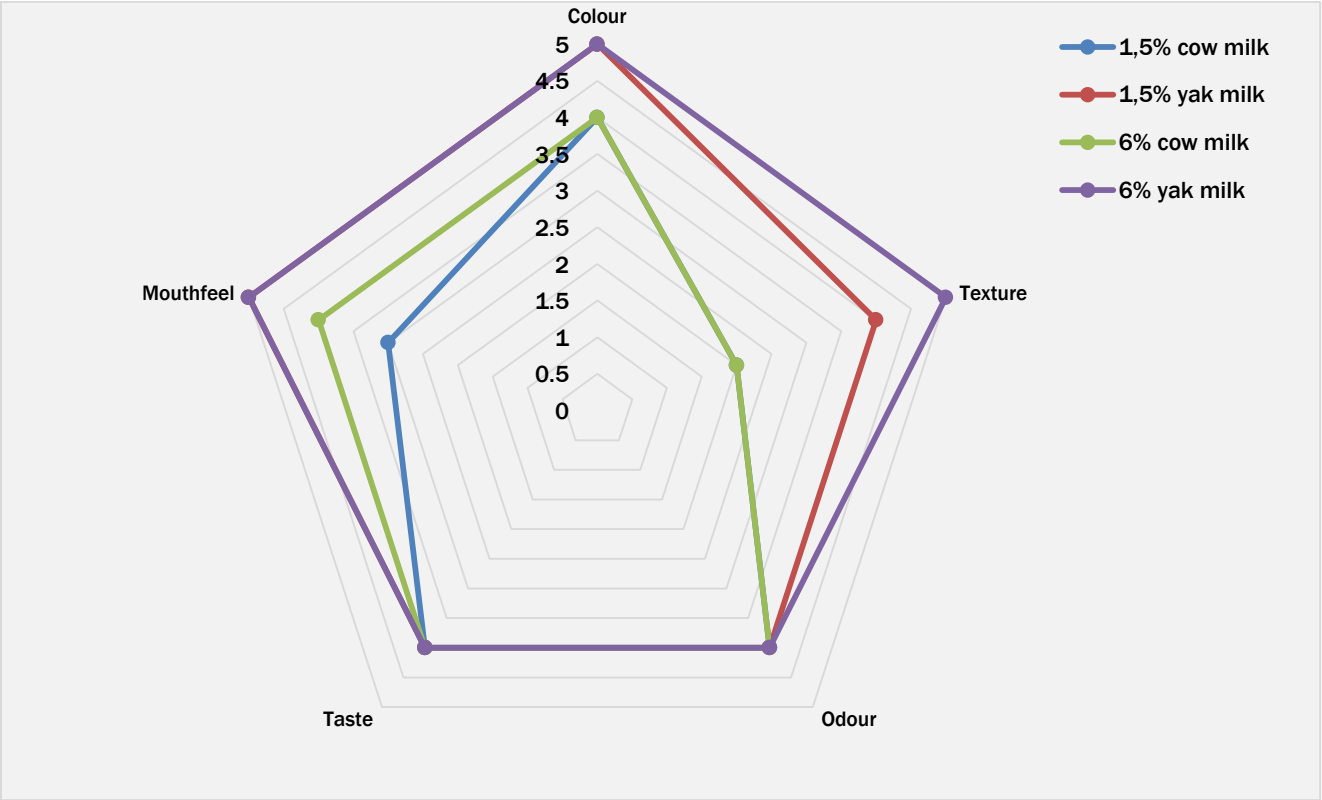

Figure 4 - Sensory analysis of fermented milk samples

CONCLUSION

The current study found that the acid-forming activity of lactobacilli manifests itself to a greater extent in yak milk, which is evident from the rate of increase in titratable acidity and the time it takes to reach the desired acidity of the final product (75 °T), which was ≈ 340 min for 6% yak milk and ≈ 540 min for 6% cow milk. That is, the duration of the technological process for producing a fermented milk drink from yak milk using the studied complex of microorganisms is reduced by 3 hours. This is due to the composition of the raw materials, characterized by a high content of dry substances, including protein. With regard to rheological properties (viscosity) of the fermented milk clot formed during the fermentation, this study revealed noticeable differences in the value of the induction period for two types of milk: 180 minutes for yak milk and 250 minutes for cow milk. By the end of the fermentation process, the viscosity of the fermented milk clot obtained from yak milk with a 6% mass fraction of fat and 1.5% is approximately 3 (93 Pa·s) and 4 (45 Pa·s) times, respectively, higher than the case is for cow milk. Organoleptic (sensory) analysis of the fermented samples confirmed 6% fermented yak milk as the best. According to the results of the research obtained in this work, yak milk is recommended for the production of yogurts and fermented milk drinks.

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DECLARATIONS

Corresponding author
Correspondence and requests for materials should be addressed to Dr. Aigul Usubalieva; E-mail: ausubalieva@manas.edu.kg

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

Authors' contributions
A. Usubalieva = organization of the experiment, literature search, discussion of the results of the analysis, writing the article, reviewing and editing; M. Musulmanova = guidance and consultations, direction of research and discussion of the results of the analysis, correction of the article; N. Saalieva = delivery of yak milk, preparation of samples, control over the fermentation process of yak and cow milk, processing of the data obtained; Z. Ozbekova = work on the rheometer, conducting, discussing, plotting and processing the rheological part of the experiment, reviewing and editing; A. Aralbek kyzy = conducting an experiment - determining titratable acidity, pH, monitoring the fermentation process of yak and cow's milk; A. Deidiev = sensory analysis of fermented yak and cow milk and tasting.

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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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ULTRASONIC-ASSISTED EXTRACTION, ANALYSIS AND IDENTIFICATION OF WATER EXTRACT OF PROPOLIS

Indha Fitria PANGESTI, Agus SUSILO, and Khothibul Umam AL AWWALY

Faculty of Animal Science, Universitas Brawijaya, Jl. Veteran, Lowokwaru, Malang 65145, Indonesia

Email: agussusilo@ub.ac.id

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ABSTRACT: *Apis mellifera* is one species of bee that produces propolis, a resin-based product. Propolis extraction using ultrasonic assistance is being widely studied. Using water as a solvent is a challenge to capture the bioactive components of propolis. This research aimed to determine the physicochemical quality resulting from the processing of propolis extract from Central Java by ultrasonics using water as a solvent at different temperatures and times. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. The study used nine treatments with three replications. The extraction time was carried out for 10, 20, and 30 minutes. The study used nine treatments with three replications. The results of the analysis showed that propolis extraction at different temperatures and times had a very significant effect (P<0.01) on the yield, total phenolic content (TPC), and total flavonoid content (TFC), with an average of 6.7–13.3%, 1.10–2.21 mg GAE/mL, and 0.07–0.32 mg QE/mL, respectively. Propolis extraction at different temperatures and times had no significant effect on tannin content, pH, and antioxidant activity. Regarding yield, TPC, TFC, and tannin content values, it was determined that extracting at high temperatures for 30 minutes produced the best results. High temperatures and long timespans are used for the best chance of collecting bioactive components.

Keywords: Bee products, Physicochemical, Processing, Propolis extract, Water solvent.

INTRODUCTION

Apis mellifera is Indonesia's most popular type of honey bee because it can adapt to tropical climates (Ustadi et al., 2021). Many of the bioactive compounds found in *Apis mellifera* propolis are polyphenols (flavonoids and tannins), phenols, and terpenoids (Cauich-Kumul and Campos, 2019). They also contain natural enzymes (carotene), antibiotics, vitamins, minerals (Al, V, Fe, Ca, Si, Mn, and Sr), and organic acids (Mammadova and Topchiyeva, 2014; Kolayli and Keskin, 2020). Besides propolis, honey bees produce honey, royal jelly, wax, and pollen products (Nur et al., 2020). The complex content of propolis can provide evidence that propolis has benefits in the food and pharmaceutical fields; Propolis possesses anti-inflammatory, antiviral, antibacterial, antioxidant, and antifungal effects (Pasupuleti et al., 2017). Antioxidant activity operates as an inhibitor, preventing reactive free radicals from oxidizing to become more stable and shielding cells from the damaging effects of free radicals (Wiwekowati et al., 2017).

Propolis can minimize the debilitating effects of heat stress in livestock by increasing intestinal crypt depth, body weight and feed intake, and immunity (Mehaisen et al., 2017; Dantas et al., 2023). Propolis has also been used as a natural supplement that can support body activities without causing adverse effects on animals and the environment (Abu-Seida, 2023). Pure propolis is not allowed to be consumed directly, bearing in mind that there are compositions in propolis that may not be consumed by humans, such as resin and wax. Propolis must be subjected to an extraction process to remove only its bioactive components for consumption.

Solvation, concentration, temperature, time, particle size, and the method utilized all impact the extraction process. Two classes of extraction methods are commonly used, namely conventional methods and modern methods. Extraction using conventional methods, for example, is the maceration method, while an example of a modern method is the UAE method, called ultrasonic-assisted extraction. Since the UAE approach is thought to save time and energy while giving strong selectivity of the targeted compounds, it is regarded as a green extraction method. It has been demonstrated to be effective in extracting several antioxidant chemicals when compared to conventional methods (Oroian et al., 2020). This technique has received much support for the present propolis extraction procedure since it is thought to be more accessible, more successful, and best for extracting propolis in terms of extraction time, extraction outcomes, and cost-effectiveness (Aboulghazi et al., 2022). In addition, compared to the maceration approach and other modern methods like the microwave-assisted extraction (MAE) method, the UAE method is more effective and yields extraction results with

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a greater propolis active component. Currently, several solvents are also used. Water is known to be one extraction method since it may extract more polar propolis components (Suran et al., 2021). However, extraction using water as a solvent still needs to be considered suboptimal in the extraction process using conventional methods. Therefore, many extraction processes are also being developed using water as a solvent, assisted by other technologies such as sonication (Sun, 2019; Dönmez et al., 2020; Contieri et al., 2023). Propolis extraction using a water solvent is also called WEP (water extraction propolis), which is more accessible due to being free of alcohol and ethanol content (Usman et al., 2016).

Based on previous studies (Yuan et al., 2019; Aboulghazi et al., 2022; Kara et al., 2022), propolis using solvents with ultrasonic assistance was able to produce higher total phenolic content (TPC) and total flavonoid content (TFC) values compared to the conventional method, namely 3.449 mg GAE/g and 0.456 mg QE/g, respectively. TPC and TFC results using conventional methods only obtained content values of 2.701 mg GAE/g and 0.336 mg QE/g, respectively (Kara et al., 2022). In addition to the type of solvent that can affect extraction, there are also temperature and time factors. The ultrasonic method can be set for temperature and time. It was stated that excessive temperature and time were also feared to damage the bioactive components. Still, if it was carried out quickly and the temperature needed higher, the compounds could not be captured optimally. The use of ultrasonics for the extraction of propolis with 60% ethanol using a frequency of 50 kHz, a power of 120 W, and a temperature of 35 °C for 15 minutes resulted in the highest TPC and TFC values and the lowest DPPH (2,2-Diphenyl-1-picrylhydrazyl), namely 187.21 mg GAE/g, 38.80 mg QE/g, and 23.70 µg/mL, respectively. Likewise, it also produces a relatively high extract yield, which is 11.25% (Aboulghazi et al., 2022). The efficiency of employing water as a solvent in the ultrasonic method for extracting propolis still needs more investigation. Based on the description above, this study identified the results of *Apis mellifera* propolis extraction obtained from Central Java, Indonesia, to see the physicochemical characteristics produced, including testing for pH, yield, TPC, TFC, tannin content, and antioxidant activity.

MATERIALS AND METHODS

Materials

Samples were prepared from raw propolis obtained from honey beekeepers in Central Java, Indonesia, provided by PT. Kembang Joyo Sriwijaya. There are 27 samples used in this study. Raw propolis samples are round and dark brown and are stored in a laboratory cupboard at room temperature. Before extraction, raw propolis is cut into small pieces to facilitate the extraction process. The solvent used was purely distilled water.

Method and statistical analysis

This study used a laboratory experimental method with a completely randomized design (CRD). Statistical analysis used a two-way ANOVA with 3x3 factorials and three replications. Furthermore, significant results were followed by Duncan's Multiple Range Test (DMRT). The first treatment factor was the use of low (A1), medium (A2), and high (A3) extraction temperatures, and the second treatment factor was the use of extraction times of 10 minutes (B1), 20 minutes (B2), and 30 minutes (B3). The temperature is observed and controlled. The low temperature used starts at room temperature with an estimated temperature of 27-30 °C, medium temperature with an estimated temperature of 40-43°C, and high temperature with an estimated temperature of 60-63 °C. The extraction method used was the ultrasonic-assisted extraction (UAE) method.

Ultrasonic-assisted extraction

The two ingredients (100 mL of aqua distillate and 10 grams of raw propolis, 1:10 ratio) were blended for 3 minutes. After blending, put it in an Erlenmeyer tube and covered with aluminium foil. The ultrasonic system used was an ultrasonic bath system with an ultrasonic frequency specification of 40 kHz and a power of 120 W. 1.5 litres of distilled water were put into the ultrasonic bath. A basket was installed to place the sample. The Erlenmeyer containing the sample was put into the ultrasonic bath and closed. Setting the temperature and time according to the treatment you want to do.

pH

pH was measured using a calibrated pH meter using pH buffers 4 and 7. The pH analysis procedure refers to the AOAC test procedure (2005). 1 mL of propolis extract dissolved in 5 mL of distilled water (1:5) was used for pH testing samples (Primandasari et al., 2021). The pH meter used is a pH meter and an EC meter (2 in 1). One mL of propolis extract was diluted in 5 mL of distilled water (1:5, v/v) and used as a sample for pH testing (Primandasari et al., 2021). The pH meter used was a pH meter and EC meter (2 in 1) (Hidayat et al., 2021). The electrode is dipped in the extract until a stable reading appears on the pH meter. The pH value results are displayed on the pH meter-monitor screen. After measurement, the pH meter was cleaned with distilled water and dried with a dry tissue before being used to collect data from the following samples. The pH electrode is immersed in the propolis extract until the pH reading on the meter stabilizes. The pH value displayed on the meter's monitor screen was then recorded. After each measurement, the pH meter is thoroughly rinsed with distilled water and dried with clean tissue before measuring the pH of the following sample.

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Yield

The percentage yield of the propolis extract was computed by dividing the weight of the freeze-dried extract by the total weight of raw propolis. The results are shown in percentages. The percentage yield is calculated following the equation (Pobiega et al., 2019):

$$\text{Yield} = \frac{\text{dry extract weight}}{\text{raw propolis weight}} 100\%$$

Total phenols content

Phenolic content was measured using UV-Vis spectrophotometry according to Lucas et al. (2022), modified. 1 mL of propolis extract was added to 2 mL of Folin-Ciocalteu reagent (0.2 M). The solution was allowed to stand for 5 minutes, then 4 mL of sodium carbonate (7.5% p/v) was added and homogenized. Until the tera mark, the homogeneous sample was mixed with distilled water. One hour was spent standing the combination at room temperature in the dark before a spectrophotometer was used to measure the absorbance at a wavelength of 760 nm. Gallic acid equivalent (GAE) was used to express the total amount of phenol obtained. Folin-Ciocalteu's Phenol Reagent was used to generate a gallic acid standard curve. Gallic acid solutions in aquadestilate were made at 0, 20, 40, 60, 80, and 100 g/mL concentrations. From each concentration, 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent were added, and the mixture was then homogenized to create a clear, yellowish solution. Eight minutes were given for the solution to stand before 3 mL of a 20% Na_2CO_3 solution was added and homogenized by shaking. Once more, the solution was left to stand at room temperature for 30 minutes until a blue tint developed. A calibration curve was created for the relationship between gallic acid content (mg/L) and absorbance after the solution's absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 760 nm (Hashim et al., 2019).

Total flavonoid content

With the help of the photometric aluminium chloride (AlCl3) method, the total flavonoid content was evaluated. The mixture of 0.5 mL of 2% $AlCl_3$ and 0.5 mL of propolis extract was homogenized and allowed to sit for 10 minutes. At a wavelength of 435 nm, the absorbance was measured using a spectrophotometer (Najafi et al.., 2007). The quercetin standard curve was made by weighing 25 milligrams of quercetin powder and dissolving it in 25 mL of distillate. To get a concentration of 100 ppm, pipette 1 mL of the solution and then add 10 mL of pure water. Then, different concentrations of a standard solution containing 100 ppm of quercetin were created, including six ppm, eight ppm, ten ppm, 12 ppm, and 14 ppm. For each concentration, pipette 1 mL of the quercetin standard solution, followed by 1 ml of the 2% $AlCl_3$ solution and the 120 mM potassium acetate solution. At room temperature, the standard quercetin was incubated for a full hour. At a maximum wavelength of 435 nm, the absorbance was calculated using UV-Vis spectrophotometry (Stankovic et al., 2011).

Tannins content

The tannin content was determined using spectrophotometry analysis. Weigh the sample to a maximum of 0.5 mL and thoroughly mix it with 5 mL of distilled water. Pipette 1.0 mL of the sample and add it to 7.5 mL aquadestilate in a 10 mL container. After adding 0.5 mL of the reagent (Folin-Denis) and letting it sit for 3 minutes, 1.0 mL of the saturated Na_2CO_3 solution was added. The absorbance was measured at a maximum wavelength of 700 nm after 15 minutes of incubation (Padey et al., 2018). Tannic acid measurement is a standard solution used to analyze total tannin content. A standard curve was used to determine the concentration of the measured sample. Standard solutions of concentrations of 10, 15, 20, 25, 30, and 35 ppm were taken in 1 ml each, and then 7.5 mL of distilled water was added. Next, 1 mL of Folin-Ciocalteu reagent was added. 1 mL of saturated Na_2CO_3 was added after the mixture had been allowed to stand for 3 minutes. The solution was kept in a dark place throughout the homogenization procedure for 15 minutes (Diniyah et al., 2023).

Antioxidant activity

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method examined antioxidant activity. 9 mL of DPPH solution was homogenized with 1 mL of propolis extract. Following homogenization, the mixture was incubated for 30 minutes at room temperature. Absorbance measurements were made using a UV-Vis spectrophotometer with a maximum wavelength of 517 nm (Hidayat et al., 2022). DPPH reagent production process 96 mL of methanol with 4 mL of stock DPPH solution (Padey et al., 2018). The solution is protected with aluminium foil and stored in a dark place. The DPPH solution, which had a concentration of 160 mg/L, was diluted with hexane to create solutions with concentrations of 4, 8, 16, and 32 mg/L. The absorbance of each DPPH solution was measured at its maximum wavelength (517 nm). The linear regression equation resulting from comparing fluctuations in the concentration of the sample against the DPPH solution was used to calculate the IC_{50} of the sample preparation against the DPPH solution. In the linear regression equation, the concentration value of the extract or the comparative antioxidant (BHT) and its inhibition % were plotted on the x and y axes, respectively. y = ax + b is the equation for the discovered linear regression. By specifying the y value of 50 and the x value to be acquired from the IC_{50}, this equation is used to determine the IC_{50} value (50% inhibitor concentration) of each sample. According to Segura-Campos et al. (2014), the IC_{50} value represents the concentration of sample solution (BHT extract or antioxidant comparator) needed to reduce DPPH free radicals by 50%.

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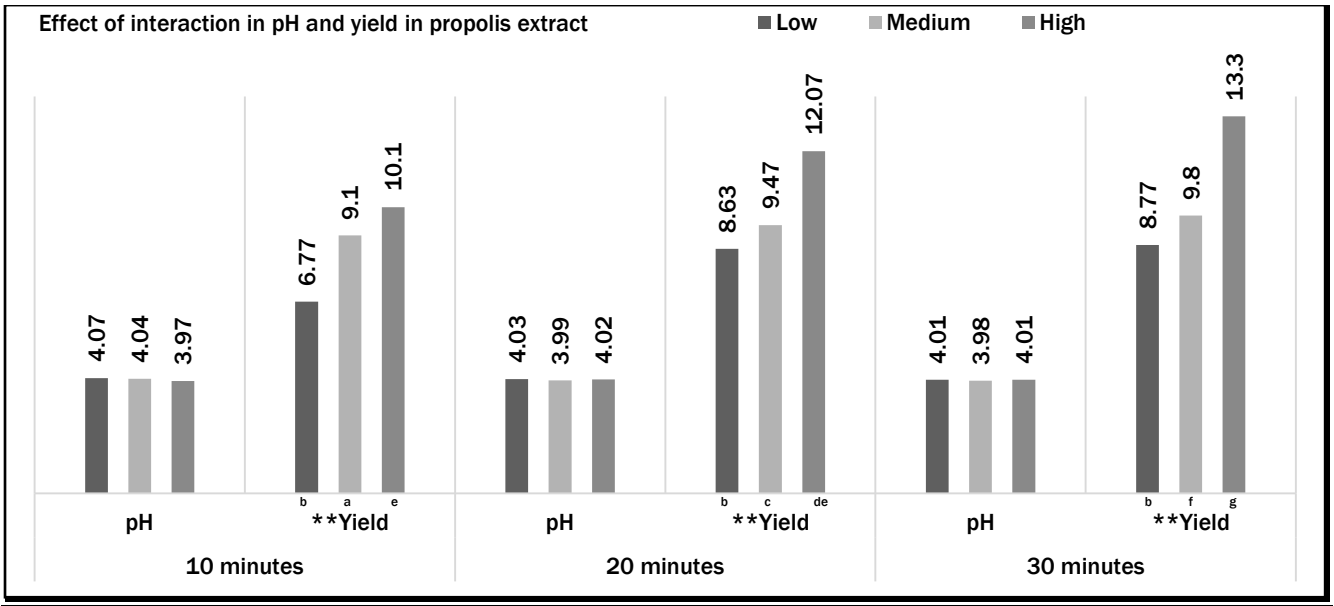
RESULTS AND DISCUSSION

Physical analysis

pH

Graph 1 and Table 2 display the findings of the analysis of the pH level of the propolis extract. The propolis extract was extracted using the UAE method at different temperatures and times, and the interaction between temperature and extraction time had no effect ($p>0.05$) on the pH of the propolis extract. The average pH value based on the interaction of the two factors is 3.97 to 4.07. The pH value shows that the propolis extract has a relatively low acidity level. The pH decreases with increasing temperature and time. Propolis' low pH value is known to prevent the growth of bacteria and fungi. Hence, propolis extract can prolong shelf life. *Apis mellifera* propolis extracted using the UAE method with a water solvent at a temperature of 35–40 °C and carried out for 5–30 minutes is known to produce a pH in the range of 3.44–3.56 (Pangesti et al., 2023). This study produced propolis extract with a slightly higher pH value, but not significantly. The acidic pH value of propolis can also be suspected because propolis contains components of organic acids and vitamin C. Besides that, it is also suspected of the presence of phenolic compounds, quercetin, and calcium. The acidity level in bee products such as propolis and honey is influenced by the plant's organic acid and mineral content, which makes the plant have distinctive characteristics (Hidayat et al., 2023). The extraction results in a lower pH as the temperature and time increase. Therefore, because the pH of the propolis extract in this study produced a low value (acid), it is suspected that there were soluble organic acid compounds. This event is linear with Oroian et al. (2020), who found that different temperature and time treatments in this study could capture organic acids and phenols in propolis, resulting in propolis extract with an acidic pH.



Graph 1 - Interaction of temperature and extraction time of the UAE method on pH and yield. **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means columns with superscripts differed significantly ($P<0.01$)

Table 1 – pH and yield with different temperature and time

	Temperature (°C)			Times (minutes)		
	Low (A1)	Medium (A2)	High (A3)	10 (B1)	20 (B2)	30 (B3)
pH	4.04±0.08	4.00±0.05	4.00±0.03	4.03±0.09	4.01±0.03	4.00±0.04
Yield (%)	$8.54±1.52^{p}$	$9.30±0.66^{q}$	$11.49±1.88^{r}$	$8.83^{x}±0.30^{x}$	$9.43±2.34^{y}$	$11.02±1.73^{z}$

p,q,r and x,y,z superscript; Means in columns with superscripts differed significantly ($P<0.01$)

Yield

The yield analysis results on propolis extract are shown in Table 1 and Graph 1. The analysis showed that the propolis extract used the UAE method at different temperatures and times, and the interaction between temperature and extraction time was very significant ($P<0.01$) on propolis extract yield. So, this research shows that the use of temperature and time significantly affects the yield produced. In addition, different temperatures and times interact with each other. This study's average extraction yield values ranged from 6.77% to 13.3%. Low extraction temperatures have not been able to produce maximum yields. However, low-temperature extraction combined with increased extraction time also

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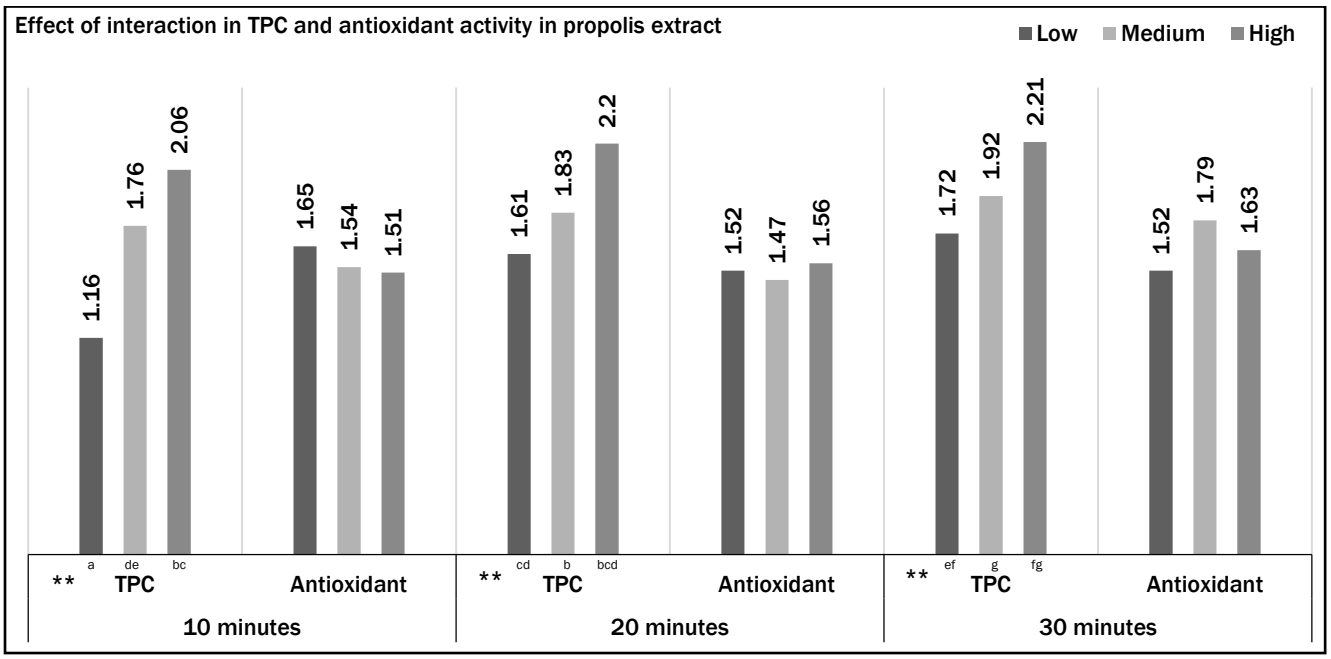
significantly affects the yield value. The increase in temperature and the length of time show an increasing extraction yield. This event shows that an increased temperature and a certain amount of time can capture more solutes. The highest yield was obtained when extraction was performed using ultrasonics at 60–63 °C for 30 minutes.

The yield results in this study tend to be lower than those of propolis extraction using the UAE method with ethanol solvent, which produces an extraction yield of 35.7–42.6% (Chong and Lee, 2020). The yield of propolis extract in this study is known to be higher than the yield of Korean propolis extraction using the UAE method with water as a solvent (Aboulghazi et al., 2022). Apart from that, Moroccan propolis using UAE water and 40% ethanol as a solvent for 30 minutes produced a yield of 8.5%. Ultrasonic-assisted extraction is known to increase yields because the cavitation bubbles produced by ultrasonics cause significant shear forces, resulting in higher extract yields. Longer extraction times can also increase extraction results because sample component degradation will take too long. However, if it is too long, it can also cause a decrease in results (Shen et al., 2023). Various studies show different yields of propolis extract, indicating that the geographical location of the sample greatly influences propolis extract because bees collect different shoots and plant exudates in the surrounding area. It can also be said that the low yield of water extract indicates that there are fewer water-soluble compounds in the propolis (Chong and Lee, 2020).

Chemical analysis

Total phenolic content (TPC)

The findings of the investigation of the total phenolic content (TPC) and IC_{50} antioxidant activity in propolis extract are displayed in Graph 2. When the proper functional derivatives are present, organic molecules called phenols containing aromatic rings are chemically linked to one or more hydrogenated substituents (Pasupuleti et al., 2017). The analysis showed that the propolis extract using UAE at different temperatures and times and the interaction between temperature and extraction time had a very significant effect ($P<0.01$) on the TPC of the propolis extract. TPC on propolis extract using low temperatures gives very different results than medium and high temperatures. The TPC results of propolis extract at different temperatures and times are shown in Table 2. The highest average total phenol was found in propolis extract extracted at high temperature, namely 2.21 mg GAE/mL, and the lowest average was found in propolis extract extracted at low temperature, namely 1.6 mg GAE/mL. Low temperatures (27–30 °C) have not been able to extract the maximum total phenol. High temperatures (60–63 °C) can produce more total phenol. Previous studies stated that using UAE at 60–65 °C for 30 minutes for propolis extraction is the optimal temperature to obtain total phenol and flavonoid content (Oroian et al., 2020). The total phenol in propolis extract is affected by temperature and the length of the extraction time, which has been observed to affect the total phenol. Extraction time for 20 minutes did not differ from extraction time for 30 minutes but was significantly different from extraction time for 10 minutes. Propolis extraction for 20 minutes resulted in a higher TPC of 1.92 mg GAE/mL compared to 30 minutes of extraction, namely 1.89 mg GAE/mL.



Graph 2 - Interaction of temperature and extraction time with the UAE method on TPC (total phenolic content) (mg GAE/mL) and antioxidant activity IC_{50} (µg/mL). **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means in columns with superscripts differed significantly ($P<0.01$).

Previous research extracted *Apis mellifera* propolis from Morocco using the UAE method with water and 40% ethanol in a ratio of 1:10 for 30 minutes, producing a TPC of 111.32 mg GAE/g (Aboulghazi et al., 2022). In contrast to the results of previous studies that also extracted Malaysian propolis using UAE at 65 °C for 25 minutes, the highest TPC content was

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0.093 mg GAE/g, while the lowest content was extracted for 55 minutes, which was 0.058 mg GAE/g. This study produced a lower TPC than Moroccan propolis but a higher TPC than Malaysian propolis, namely Central Java propolis, which produced a TPC of 1.16 to 2.21 mg GAE/mL. The phenolic compound content of propolis extract in each country is thought to be because the chemical content of propolis extract is also influenced by differences in regional origin and surrounding plant vegetation, as well as climatic factors (Lim et al., 2023). Water is a more polar solvent than solvents with a mixture of 60% and 80% water and ethanol, which are known to extract more polar compounds. A higher polarity allows the extraction of many binding compounds from the propolis material, which has relatively more polar properties. Phenolic compounds are known to be primarily soluble in polar solvents. Phenolic compounds are phenols and include flavonoids, tannins, and alkaloids. The increasing extraction temperature results in the total phenol value increasing, which can be expected because the high temperature used during extraction can reduce the viscosity of the solvent so that it can increase the penetration ability of the solvent into the propolis mass, which increases extraction efficacy (Suran et al., 2021; Sasongko et al., 2017). The extraction in this research, which utilizes water as a solvent and is supported by ultrasonic methods with increased temperature and time, can produce significantly increased phenolic compounds.

The phenol results are associated with a pH value known to have a low pH (acid), which shows that the extraction process using polar water assisted by ultrasonics can produce propolis extract with a high phenol content. Using a higher sonication temperature will cause changes in vapour pressure, surface tension, viscosity, and solvent, thereby affecting the extraction cavitation process and causing cell wall damage, which can then increase the diffusivity of phenolic compounds. High temperatures can also increase solubility, thereby speeding up the extraction process. High extraction temperatures are only sometimes suitable in the UAE because phenolic compounds are heat-sensitive. It can be concluded that extraction using temperatures up to 100 °C will be expected to reduce the total amount of phenol (depending on the solvent's boiling point). Suppose the heat of sonication exceeds the boiling point of the solvent. In that case, more and more of the solvent will be evaporated, causing the volume to continue to decrease, thereby reducing extraction efficiency (Yusof et al., 2020). Based on the results of the total phenol content in this study, using the highest temperature was considered good because it did not exceed the solvent's boiling point and showed increasing results at temperatures of 60–63 °C.

Antioxidant activity as IC_{50}

The DPPH method, which measures the amount of the reduction in the absorption of free radicals in DPPH solutions at a wavelength of 517 nm, was used to test the antioxidant activity of various substances. The antioxidant activity parameter uses IC_{50} (the initial DPPH concentration by 50%), which is the concentration of the extract (fraction) that contributes 50% antioxidant activity compared to the control through the linear regression line equation (Wiwekowati et al., 2017). There are five classifications of IC_{50} values to determine their strength: a powerful antioxidant group is one with an IC_{50} value of 50 g/mL; a potent antioxidant group is one with an IC_{50} value of 50 to 100 g/mL; a moderate antioxidant group is one with an IC_{50} value of 101 to 150 g/mL; a weak antioxidant group is one with an IC_{50} value of 15 to 200 g/mL; and a frail antioxidant group (Hidayat et al., 2022). The statistical analysis results in this study showed that propolis extract using UAE at different temperatures and times had a significant effect ($p>0.05$) on the antioxidant activity IC_{50}. The average IC_{50} value was obtained in the 1.47-1.79 µg/mL range. Low temperatures produce the lowest IC_{50} value compared to medium and high temperatures. Extraction time for 20 minutes produces the lowest value compared to extraction time for 10 and 30 minutes, as shown in Table 2. The interaction between temperature and time that produces the lowest IC_{50} value is obtained using a medium temperature (40–43 °C) for 20 minutes, as much as 1.47 µg/mL. The DPPH antioxidant activity was observed to have an IC_{50} value <50 µg/mL; this shows that the antioxidant activity in *Apis mellifera* Central Java propolis extract has intense activity. Extraction using different temperatures and times, along with the interaction between the two, does not influence the IC_{50} value, but using a higher temperature with a longer extraction time can potentially increase the IC_{50} value.

Romanian ethanol propolis extracted using UAE produced an average IC_{50} value ranging from 0.0700 to 0.9320 mg/mL. In addition, Malaysian propolis extracted using UAE with water and acid ethanol solvents produced an IC_{50} of 0.1731 mg/mL (Chong and Lee, 2020). This event shows that Central Java propolis extract with distilled water solvent produced a lower IC_{50} value, so it has more robust antioxidant activity than Romanian and Malaysian ethanol propolis. However, this study showed that extraction with temperatures reaching 60 °C and times exceeding 20 minutes reduced antioxidant activity. UAE produced an acoustic cavitation effect that can cause high temperatures to reduce particle size and increase mass transfer. Therefore, the UAE method only requires a short time and low amounts of solvent (Bankova et al., 2021). Based on the research of the relationship between temperature utilization and extraction time, the lowest IC_{50} was obtained from propolis extraction using a moderate temperature (40–43 °C) for 20 minutes. These antioxidant properties are usually directly related to the total phenol content. It was proven in this study that propolis extraction using UAE with water as a solvent could bind phenolic compounds in Central Java propolis, so it could also detect the presence of its antioxidant activity.

The analysis of antioxidant activity in this study used a standard solution of gallic acid as a comparison because gallic acid is known to have strong and stable antioxidant properties. The results of the antioxidant activity study illustrate the ability of total phenols in propolis extract to act as antioxidants. However, based on the statistical analysis results, total phenol is not directly proportional to antioxidant activity. It is suspected that the antioxidant activity in propolis extract comes from total phenols and the interaction of several other phenolic compounds, such as tannins and

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flavonoids, which also have antioxidant activity. Phenolic compounds are known to contribute to antioxidant activity because they can donate hydrogen atoms or electrons to free radicals to bind free radicals and decompose oxidation products (Diniyah and Lee, 2020). Although the antioxidant activity of DPPH in this study did not provide a significant difference, it appeared to have a relationship with total phenols and total flavonoids, so it was able to produce low IC_{50} values (Cottica et al., 2011).

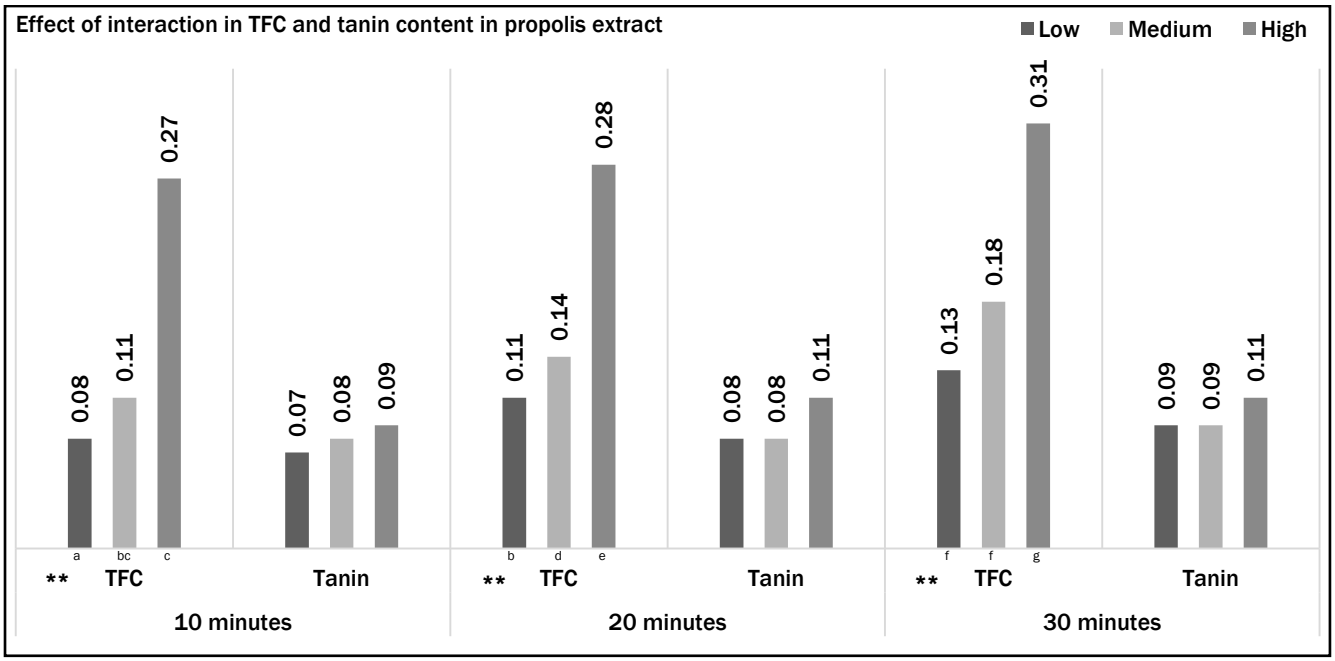
Table 2 –TPC, TFC, tannins content, and antioxidant activity with different temperature and time

Parameters	Temperature (°C)			Times (minutes)		
	Low (A1)	Medium (A2)	High (A3)	10 (B1)	20 (B2)	30 (B3)
TPC (mg GAE/mL)**	1.60±0.35^{p}	1.73±0.11^{q}	2.16±0,10^{r}	1.68±0.41^{x}	1.92±0.27^{y}	1.89±0.23^{y}
TFC (mg QE/mL)*	0.11±0.02^{p}	0.14±0.03^{q}	0.29±0.02^{r}	0.15±0.09^{x}	0.18±0.08^{y}	0.21±0.08^{z}
Tanin content (mg TAE /mL) *	0.08±0.01^{p}	0.09±0.01^{p}	0.10±0.01^{q}	0.08±0.01^{x}	0.09±0.01^{y}	0.10±0.01^{y}
Antioxidant activity IC_{50} (µg/mL)	1.56±0.39	1.60±0.15	1.57±0.08	1.57±0.08	1.52±0.38	1.65±0.13

**p,q,r and x,y,z superscript; Means in columns with superscripts differed significantly (P<0.01). *p,q and x,y superscript; Means in columns with superscripts differed significantly (P<0.05); TPC: Total Phenolic Content; TFC: Total Flavonoid Content; QE= quercetin equivalent; GAE= Gallic acid equivalent.

Total flavonoid content

Graph 3 shows the results of determining the total flavonoid (TFC) and tannin content. Flavonoids are a derivative of phenolic compounds with a conjugated aromatic ring system so that they can absorb UV-vis. The analysis demonstrated that the propolis extract was made using a UAE process with various extraction times and temperatures. As well as the interaction between temperature and time had a very significant effect (P<0.01) on the total flavonoids of the propolis extract. Follow-up tests showed that the total flavonoids of Central Java propolis extract extracted at low temperatures significantly differed from medium and high temperatures. The highest TFC average was obtained in propolis extract that was extracted at a high temperature, which was 0.28 mg QE/mL, and the lowest average was found in extraction that used a low temperature, which was 0.11 mg QE/mL. Low temperatures (27–30 °C) have not been able to extract flavonoids maximally, as shown in the observations in Table 2, as well as the extraction time of 10 minutes. The amount of TFC produced likewise rises as the temperature rises. This condition also holds for the utilization of extraction time; the higher the TFC, the longer the time used. It is demonstrated that the TFC in propolis extract is influenced by both temperature and the duration of extraction. The interaction between temperature and extraction time is also significantly related. Along with the high temperature (60-63 °C) and the long time used, the highest TFC was 0.31 mg QE/mL. The increasing temperature and length of time used show that the total phenol also increases.



Graph 3 - Interaction of temperature and extraction time with the UAE method on TFC (total flavonoid content) (mg GAE/mL and tannins content (mg TAE/mL). **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means columns with superscripts differed significantly (P<0.01)

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Ethanol is a solvent commonly used to dissolve flavonoid compounds (Azmir et al., 2013; Pandey and Shalini, 2014). In general, water solvents can only dissolve polyphenols and phenolic compounds (Stalikas, 2007). However, this study proved that extraction using a water solvent with ultrasonic assistance can dissolve flavonoid compounds even at low values, following the statement of Khoddami et al. (2013) that ultrasonic-assisted extraction can help extract phenolic and flavonoid compounds. Ultrasonic waves cause damage to the cell walls, which causes the cell contents in the form of plant metabolites to come out. Ultrasonic-assisted extraction in this study could bind flavonoids even though it only used water as a solvent. Research by Aboulghazi et al. (2022) extracted *Apis mellifera* propolis from Morocco using UAE with 40% water and ethanol as a solvent and the same ratio (1:10) for 30 minutes, producing a total of 34.72 mg QEq/g flavonoids. Based on the results of this research, use of time for 30 minutes showed a lower average total flavonoid result, namely 206.38 µg QE/mL or 0.21 mg QE/mL. The total amount of flavonoids in the study was still below Moroccan propolis. Still, it was relatively higher compared to the results of previous research that used Malaysian propolis, which was extracted using UAE for 30 minutes with 70% distilled water-ethanol solvent, namely only 0.015 mg QE/mL or 150 µg QE/mL (Zainal et al., 2021). The flavonoid content in propolis was higher when extracted using the UAE method (Bankova et al., 2021). The total flavonoids produced in Central Java propolis extract are higher as the extraction time used increases. The longer the UAE extraction time, the more compounds it will produce. It was learned in previous research that increasing the extraction time to more than 30 minutes will reduce total phenolic and total flavonoid compounds because it can cause compound degradation. Cavitation bubbles will burst so that they can damage the substances in the solution. Total flavonoids and total phenols were studied to influence the antioxidant (antiradical) and reduce the activity of propolis (Bouaroura et al., 2019).

Tannins content

Tannins are known as a type of secondary metabolite compound that can be found in plants. In addition, tannins are polyphenols that can react with extracellular enzymes and bacterial cell walls. By blocking the entry of nutrients into cells, this technique can stop the growth of these bacteria. Tannin compounds can dissolve in water solvents (Lim et al., 2023). The analysis showed that the Central Java propolis extract used the UAE method at different temperatures and times, and the interaction between temperature and time had no effect ($p>0.05$) on the tannin content of the propolis extract. A table of the analysis of the effect of using different temperatures and times has been presented in Table 2, and the interaction between temperature and extraction time is shown in Graph 3. The results of further tests showed that the tannin content in propolis extract extracted at low temperatures was not different from that at medium and high temperatures. The average tannin content of Central Java propolis extract ranged from 0.07 to 0.11 mg TAE/mL. Extraction at a low temperature for 20 minutes and 30 minutes produced the same tannin content as extraction at a low temperature for 10 minutes. This condition shows that low temperatures can produce tannin content, and an increase in temperature does not significantly affect the resulting tannin content. However, the results show quite a visible difference as the extraction time increases.

The lowest tannin content was obtained at a low temperature for 10 minutes, while the highest was obtained at a high temperature for 30 minutes. Increasing the temperature and length of time can also result in increased tannin levels, which indicates that the levels of secondary metabolites carried by the solvent increase. The secondary metabolite compounds in propolis are tannin compounds (Chong and Chua, 2020). Previous research studied that extraction using UAE at a temperature of 55 °C produced higher tannin levels than 35 °C. Increasing temperature can increase mass transfer, affecting the observed extraction (Padey et al., 2018). The results of the tannin content of propolis extract in this study accumulated positively with total flavonoids. It is plausible since tannins are part of the total flavonoids. Tannin is also thought to be one element that gives propolis its dark colour (Lim et al., 2023). Propolis tannin levels are rarely discussed in research. Previous studies on conventionally extracted Central Java propolis detected a tannin content of 0.213%, but the concentration of tannins was less than the tannins in South Sulawesi propolis, which was 0.957%. Seven different kinds of plants can produce resin; these include durian, cempaka, cocoa, pine, randu, resak, and cassava. According to Mahani et al. (2021), these tannin compounds are known to have antibacterial and antioxidant effects.

CONCLUSION

This research can evaluate the results of the content of bioactive compounds, such as phenols, flavonoids, and tannins, in *Apis mellifera* Central Java propolis extract. Propolis extraction using a water solvent with ultrasonic assistance produces good physical and chemical quality, although it is still lower than some literature results. Central Java propolis extract (WEP) obtained maximum results with treatment at a temperature of 60–63 °C for 30 minutes in ultrasonics in terms of extract yield (13.3%), TPC (2.21 mg GAE/mL), TFC (0.31 mg QE/mL), and tannin content (0.11 mg TAE/mL). The UAE method is proven to be able to help the extraction process in a shorter time; however, propolis extraction using water as a solvent is too short, and at low temperatures, it is still not optimal for producing bioactive components, especially phenols, flavonoids, and tannins. Based on the research results, use the lowest temperature of 60 °C for 30 minutes to obtain maximum propolis extract.

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DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Agus SUSILO; E-mail: agussusilo@ub.ac.id; ORCID: https://orcid.org/0000-0002-4440-5806

Data availability

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

Authors' contribution

IF. Pangesti, A. Susilo, and K.U.A. Awwaly contribute to the research, data analysis, and manuscript writing.

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Consent to publish

The authors agree to the publication of this manuscript.

Competing interests

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

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ISOLATION AND MOLECULAR IDENTIFICATION OF THE *invA* GENE OF *Salmonella* spp. IN DROMEDARY CAMELS

Ali Kadhim ALTAEE and Afaf Abdulrahman YOUSIF

Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, P.O. Box 28601 AlDawoodi, Iraq

Email: afaf.a@covm.uobaghdad.edu.iq

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ABSTRACT: This study was done to determine the percentage of *Salmonella* spp. in camels from three provinces (Karbala, Al-Najaf and AL-Muthana) in Iraq with different age and both sexes. Total of 250 fecal samples from 250 camels were collected. Diagnostic study depended upon the morphological and cultural properties of the isolates on some selective media like Xylose lysine deoxycholate (XLD) and *Salmonella Shigella* (SS) agars which were used in addition to different biochemical tests and molecular assay by PCR for detection of virulence gene invasion A (*invA*) with Phylogenetic study. The clinical signs appearing on animals infected with *Salmonella* were greenish diarrhea, loss of appetite with mild systemic reaction. Bacteriological and molecular tests revealed isolation of five *Salmonella* isolates with *invA* gene. Two of these isolates were sequenced. The results showed that the first strain *S. enterica subspecies typhimurium* (LC730846) converged with a group of global strains with one node, as it converged with the global strain that held the clade (MK017934.1 and MT460418.1). While the second local strain *S. enterica serovar enteritidis* (LC730849) appeared with a new node and it is not affiliated with any association with the world *S. enterica* strains. It is concluded that the presence of *Salmonella* spp. in camels needs monitoring in order to minimize the risks of infection exposing human beings.

Keywords: Camels, Fecal samples, *invA* gene, PCR, *Salmonella*.

INTRODUCTION

Salmonellosis is an important infectious zoonotic disease that affects the health and economic effect of industrialized and developing countries. In developing nations, salmonellosis is often a common but neglected disease (Mohammadpour et al., 2020). Members of genus *Salmonella* are ubiquitous in nature. They live in the human (Abebe et al., 2020), various animals (Afaf et al., 2010; Al Zubuidy and Yousif, 2012), animals such as goats, dogs and cows (Afaf et al., 2010; Al Zubuidy and Yousif, 2012) respectively and Pets contaminate the environment of their owners by shedding *Salmonella* intermittently in their feces (Dróżdż et al., 2021).

Salmonellosis is characterized by enterohepatic or enteric manifestations, which result in several clinical signs, that included neonatal diarrhea abortion, orchitis, pneumonia, and septicemia (Abdelwahab et al., 2019). Chronic salmonellosis in camels is characterized by diarrhea, weight loss and death within a few weeks, Humans can be infected by the consumption of contaminated foods originated from camels, infected drinking water, or close contact with infected camels (Wernery and Kaaden, 2002).

Salmonella infection of camels is reported in Sudan, UAE, Palestine, France, Somalia, North Africa, USA, Ethiopia, Egypt, and Iran (Sepehr, 2012). Sevilla-Navarro et al. (2021) reported that the prevalence of *Salmonella* in camels was 5.5% (3/54), and the only serovar isolated was *S. Frintrop* Using pulsed-field gel electrophoresis analysis which revealed low genetic diversity, and all isolates showing nearly identical pulsotype (similarity >95%), they indicate that dromedary camels seems to be a reservoir for *Salmonella* transmission especially camel riding has become as one of the main touristic attractions in different countries which increases the popularity in recent years.

The pathogenesis of salmonellosis was begins with the local colonization of bacteria, followed by bacterial spread of infection to other regions of body, which leads to different clinical signs according to the virulence of *Salmonella* strain (Robert et al., 2013). Twenty eight serotypes of *Salmonella* were isolated from camels in United Arab Emirates by Winery (1992), these serotypes were identified with the most frequent *S. saintpaul*, followed by *S. frintrop*, *S. hindmarsh* and *Salmonella typhimurium*.

Camels are an important source of *Salmonella*. It is, therefore, important to control and prevent salmonellosis in these animals and their products to decrease the transmission to a human. Therefore, this study aimed at isolating and identifies *Salmonella* species in camels with determining their virulence gene *invA* via conventional PCR assay with phylogenetic analysis.

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MATERIAL AND METHODS

Ethical committee

This study was approved by the ethical and research committee of Veterinary Medicine of College, University of Baghdad, book No. 39/D A in 7/12/ 2021.

Animals and samples

The study was performed on 250 camels at field located in three provinces in Iraq [Karbala (36), AL Najaf (191) and AL Mothana (23)]. Camels aged from one day -seven years, and were from both sexes. The study extended from January 2021 to December 2021. Data and history obtained from owner, clinical examination are recording, systemic reaction (temperature, pulse, respiratory rates), and presence or absence of diarrhea and other signs (Al-Graibawi et al., 2021). 250 fecal samples were collected from these camels and put in sterile container and kept on ice till reaching the laboratory.

Isolation and identification of *Salmonella*

Fecal samples (1 gram) were transferred in sterile container immediately to the lab in ice box. The samples were cultivated on Selenite-F Broth and MacConkey agar and incubated aerobically at 37 °C for 24- 48 hours. Microscopical examination by Gram staining was done according to Quinn et al. (2004) and the bacterial cells were examined using X100 lenses with immersion oil. After cultivating suspected colonies on Xylose lysine deoxycholate (XLD) agar and *Salmonella Shigella* (SS) agar was confirmed by Gram staining and different biochemical tests (oxidase, urease, indole tests, catalase, TSI and citrate utilization).

Antibiotic susceptibility test

Antibiotic susceptibility of isolates was determined by disc diffusion method (Bauer et al., 1966) on Muller Hinton agar. The test was done by using different antibiotic discs according to (Quinn et al. 2004, Arcan and Afaf, 2013). The antibiotic inhibition zone was estimated as mention the Clinical and Laboratory Standards Institute (CLSI, 2023).

Molecular assay: PCR and sequencing

Primers used in this study were obtained from Bioneer Company, Korea and were designed based on the sequence of the *invA* gene: Forward primer GTGAAATTATCGCCACGTTCGGGCAA and reverse primer TCATCGCACCGTCAAAGGAACC with an expected amplicones size of 280 bp (Cocolin et al., 1998).

Primer	Primer /sequence 5- to 3-		Product size (bp)
InvA	F	GTGAAATTATCGCCACGTTCGGGCAA	284
	R	TCATCGCACCGTCAAAGGAACC	

DNA extraction and PCR program

The isolation of DNA genomic from bacterial growth done according by using DNA extraction kit (Addprep bacterial genomic DNA extraction kit) from Addbio Company (Korea). The purity and concentration of the final template DNA after extraction were measured by Quantus Fluoro meter (Promega Company, Korea). Colonies suspected to be *Salmonella* by conventional phenotypic methods were placed in 500 µl of distilled water and boiled for 10 min to release the genomic DNA and subsequent detection of the *invA* gene by PCR. The strains were stored at -70°C for their future sequencing analysis. The PCR reaction cocktail was contained of 50 mM KCl, 10 Tris-HCl mM pH 9, 0.1% Triton X-100, 2 mM MgCl2, 0.01% of gelatin, 0.2 mM of each dNTP, 1 µM of *invA* primer (Bioneer, Korea), 1U of Taq DNA polymerase (Highway®) and 5 µl of DNA. The thermal profile used for the detection of the *invA* gene was detailed in (Table 1). The PCR were analyzed by electrophoresis in a 2% agarose gel in the presence of ethidium bromide. The band size was determined by comparing the products amplified with the molecular size marker DNA Ladder 100 bp (Promega®, Korea).

Table 1 - PCR thermocycler condition of *invA* gene

Thermocycling	Primer / *InvA*	Cycles
1- Initial denaturation	95°C / 5min	1
2- Denaturation	95 °C / 30 sec.	35
3- Annealing	60°C / 35 sec.	35
4- Extension	72 °C / 55 sec.	35
5- Final extension	72°C / 5 min	1
6- Hold	4°C	

Sequence analysis

All samples with positive PCR product (20 µl) were sent to (Macrogen company, Korea) for sequence analysis by using sanger macrogen analyzer for determination sequence variation among the isolates, the results was analysis by using Bioedit software version (3.1) and phylogenetic tree analysis was performed by mega 11.0. Based on the NCBI-BLAST data, Multiple Sequence Alignment Analysis of *invA* gene, phylogenetic tree, and homology sequence identity were made with the Genbank-NCBI strain/isolate (Gharban and Yousif, 2020).

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Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference–LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

RESULTS

Clinical signs

Clinical signs included diarrhea with greenish color feces (Figure 1), and with yellowish to white color (Figure 2). Other clinical signs were loss of appetite, moderate dehydration, dullness, congestion of mucous membranes and slight increase of temperature, pulse and respiration rates.

Figure 1 - Camel affected with *Salmonella* showed diarrhea with greenish color, recombanacy and fever.

Figure 2 - Camel affected with *Salmonella* showed diarrhea with yellowish to white color with dehydration.

Isolation of *Salmonella*

From 250 fecal samples collected from diarrheic and non-diarrheic camels, 5 isolates of *Salmonella* (2 %) were determined. Cultivation characteristics of *Salmonella* spp. isolates showed colorless, smooth colonies on MacConkey agar, On *Salmonella Shigella (SS)* agar the organisms were produced small sized with black pin-head, circular or round smooth, raised, colorless (Figure 3A), while the colony was pale pink with large black center on Xylose Lysine (XLD) agar (Figure 3B). The colony on smears on Gram's stain showed G-ve bacteria pink in color, the shape appeared as small rod under the microscopic. To classify the isolates of bacteria, a number of biochemical tests were conducted on *Salmonella* isolates as shown in (Table 2).

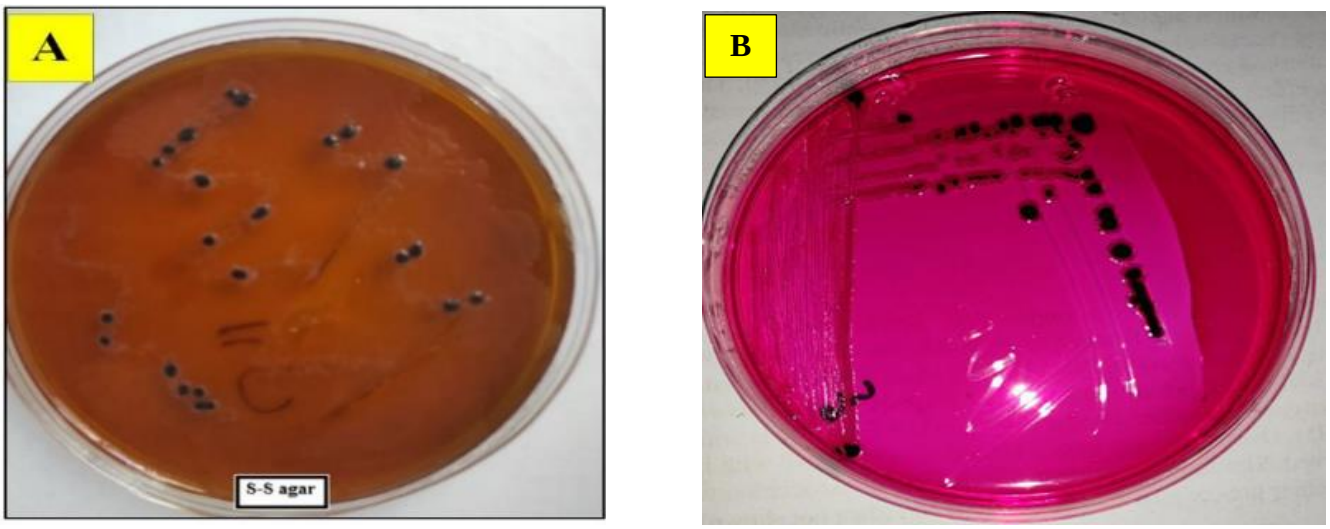

Figure 3 - Colony of Salmonella on SS agar (A), on XLD agar (B).

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Table 2 - Results of *Salmonella* isolates on biochemical tests.

Tests / Bacteria	Oxidase	Catalase	Indole	VP	Citrate	H_2S	Motility	Urease
Salmonella	-	+	-	-	+	+	+	-

Antibiotic succebitbilty of *Salmonella* spp.

The antibiotic susceptibility of *Salmonella* spp. is presented in Table 3 and Figure 4. The results revealed that the 5 (100%) of *Salmonella* spp. isolates were resistant at least 9 antibiotic; Gentamicin, Erythromycin, Tetracycline, Ciprofloxacin, Amikacin, Sulfamethoxazole, Trimethoprim, Nalidixic acid and Cefotaxime The 5 *Salmonella spp.* isolates revealed susceptibility to 4 antibiotic classes.

Table 3 - Antibiotic drugs against *salmonella spp.* isolates. S: sensitive isolates, R: resistant isolates to antibiotic

Antibiotic (concentration)	S (%)	R (%)	P-value
Ampicillin (10 µg)	12 (100%)	0 (0%)	
Amoxicillin (30 µg)	12 (100%)	0 (0%)	
Gentamicin (30 µg)	0 (0%)	12 (0%)	
Erythromycin (60 µg)	0 (0%)	12(100%)	
Tetracycline (5 µg)	0 (0%)	12(100%)	
Ciprofloxacin (10 µg)	0 (0%)	12(100%)	0.0037 **
Amikacin (10 µg)	0 (0%)	12(100%)	
Sulfamethoxazole (30 µg)	0 (0%)	12(100%)	
Trimethoprim (30 µg)	0(0%)	12 (100%)	
Chloramphenicol (30 µg)	12(100%)	0(0%)	
Nalidixic acid (30 µg)	0 (0%)	12(100%)	
Cefotaxime (25 µg)	0 (0%)	12(100%)	
P-value	0.0074 **	0.0069 **	—

**: P≤0.01.

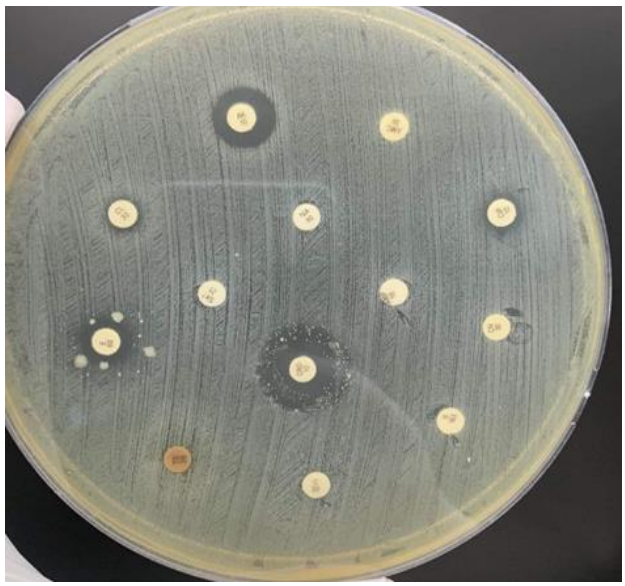

Figure 4 - The antibiotic succebitbilty of *Salmonella*

Results of molecular assay

In PCR technique, all the *Salmonella* isolates amplified a 248 bp DNA amplicones, which suggested the presence of *invA* and further confirmed that all the isolates were *Salmonella.*

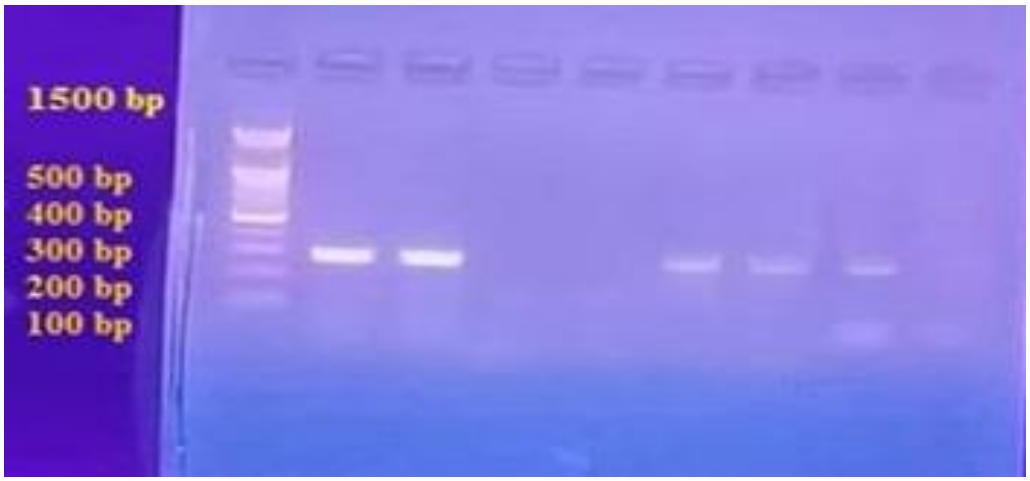

Figure 5 - Representative Agarose gel analysis of PCR assay targeting invA gene in *Salmonella* isolates. Lane M: 100 bp DNA ladder, Lane 1: Negative control, Lane 2 3, 6, 7, and 8): *InvA* gene PCR results.

Identification of clades in phylogenetic tree

Present results obtained two serotypes of *Salmonella* namely *Salmonella enteritidis* and *Salmonella typhimurium* by sequencing analysis with two accession number from gene bank websites as (LC730849, LC730846) respectively (Figure 6).

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Figure 6 - phylogenetic analysis of *Salmonella spp*. Among different isolates in different countries.

DISCUSSION

There are various studies showing the prevalence and seroprevalence of *Salmonella* spp. in camel farms, as well as the risk factors associated with the presence of the bacterium and the prevalence in slaughter house (Wernery, 1992; Bosilevac et al., 2015; Sevilla-Navarro et al. 2021).

The criteria for the cultural and biochemical identification of *Salmonella* spp. are widely described and the detection of *Salmonella* spp. by conventional culture is considered the reference method (AL-Darraji and Yousif, 2012). However, this method suffers from the disadvantage of the time required for the obtaining a result, these tests depend on the appearance of the analyzed characteristics and can be affected by variations in culture media and in the incubation conditions. Alternatively molecular methods are being used more and more; allowing a faster and simpler diagnosis (Tracogna et al., 2013)

Currently, *Salmonella* is detected by standard bacteriological, biochemical and serological techniques. These techniques are generally time-consuming, tedious and expensive as they require hundreds of antisera as well as well-trained technicians (Nora and Thong, 2010). Many researchers underlined the importance and necessity of founding a more rapid and effective detection methods as a basis of controlling the infection (Al-Zubaidy et al., 2015). Several rapid and sensitive methods have been developed for identification of Salmonella serotypes from clinical specimens (Salih and Yousif, 2018a).

All strain of Salmonella isolates were resistance to many antimicrobial agents, the findings of the current study were in accordance with Kipper et al. (2019) who found (100%) *Salmonella enterica* isolate carrying this region of resistance in the petri dish among 63 isolates. Lekagul et al., (2020) mentioned that the *Salmonella* spp. have the ability of spread the antibiotic resistance by the genes called transfer-associated, thus, so causing increase in the incidence and the severity of the disease. Also the treatment of *salmonella* in all animals was difficult due to multidrug- resistant of *Salmonella* spp.

Salmonella specific PCR with primers for *invA* is rapid, sensitive and specific for detection of *Salmonella* in many clinical samples. The detection of a 284 bp product of the *invA* gene, specific for *Salmonella* spp., was achieved by using the conventional PCR variant. This PCR technique has been shown to be reproducible, specific and sensitive for the detection of *Salmonella* spp. It is recommended that the standardization of a PCR be carried out in each laboratory. The

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qualitative comparison of the conventional PCR assay with bacteriological culture showed a greater efficiency of PCR in terms of sample processing time and the time needed for obtaining results (36 to 48 hours), compared to the culture technique (5-6 days).

The sequencing of the product obtained confirms that the PCR method detects the *invA* gene of different serovar of *Salmonella enterica*, which guarantees the specificity of the PCR assay and is also a requirement for the validation for the diagnosis of pathogens in different clinical isolates.

InvA is a gene that is shared by all *Salmonella* species; as a result, it is frequently employed as a genetic target that is selective for the detection of *Salmonella* strains (González-Escalona et al., 2012). Furthermore, it has been reported that *invA* has mutation rates comparable to housekeeping gene indicating that it is a potential candidate pertaining to tests for detection using PCR (Boyd et al., 1997). invA sequences that can be obtained from the NCBI (23 global unique sequences, with 2 additional local *S. enterica subsp. enterica typhimurium* and *S. enterica subsp. Enterica enteritidis* that were sequenced throughout the course of this research, due to the fact that sequences for these serovar were not readily accessible to the public. A phylogenetic tree was generated using the maximum likelihood method with the core genome sequences of 23 reference strains; nucleotides clustering, sequence type, serotype, country, and source are indicated at phylogenetic tree, *Salmonella* spp. isolates belonged to the following species and serovar and accession number: *S. enterica serovar Enteritidis* (LC730849) and *S. enterica subspecies typhimurium* (LC730846).

CONCLUSION

In conclusion, the PCR technique is a basic tool for the detection of *Salmonella* enterica, and its virulence factors, such as the *invA* gene, provides information ranging from the identity of a given bacterium to its virulence potential, and Sequencing of *invA* gene of *Salmonella* showed identity 99%-100% with the world level. Also, it is suggested that the presence of *Salmonella* spp. in camel needs monitoring in order to minimize the risks of infection exposed the human beings.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Afaf Abdulrahman Yousif; Email: afaf.a@covm.uobaghdad.edu.iq

Data availability

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

Authors' contribution

All authors contributed equally in all details of this paper.

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Consent to publish

All authors agree to publish the article "Isolation and molecular identification of *invA* gene of *Salmonella* spp. in dromedary camels" in an online journal of animal and feed research.

Competing interests

The authors declare no competing interest.

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PRODUCTIVITY OF THE TSIGAI SHEEP BREED UNDER DIFFERENT FEEDING REGIMENS

Alla KITAEVA, Vira MAMEDOVA, Olena BEZALTYCHNA, Ihor SLYUSARENKO, and Alyona NOVICHKOVA

Department of Technology of Production and Processing of Animal Husbandry Products, Odesa State Agrarian University, str. Pateleimonivska, 13 Odessa, 65000, Ukraine

Email: mamedova_vera@ukr.net

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RESEARCH ARTICLE

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ABSTRACT: In the present study, the influence of levels of feeding on the formation and development of economic and commercial traits of the Tsigai breed was studied in the conditions of the southern steppe of Ukraine. The research was conducted on purebred sheep from birth to 14 months of age. For this purpose, 2 groups of 3.5-4 years old ewes of the first class were selected with 40 heads in each class. It was established that poor feeding of ewes (experimental diet and below standard nutritional levels) and their offspring at the early stage of ontogenesis had a negative effect on the formation and growth of productive qualities of lambs, means of live weight and wool productivity indicators. Qualitative and quantitative indicators of wool were better in ewes obtained from mothers of the control group (who received a balanced diet in accordance with the standard of feeding). Advantage in length of wool at 12 months age was 29.3%, shearing of unwashed wool (26.7%), washed (26.5%), strength of wool at 4 months of age (10.5%), and in the 14th month aged was 5%. The improvement in housing and nutrition conditions in the control group proved that the counts were better and this had a very positive effect on the productivity of the sheep. In conclusion, full-fledged feeding of ewes of the Tsigai breed ensured good development of offspring at all stages of ontogenesis and contributed to the birth of healthy, viable lambs that are capable of high productivity. Any decline in nutrients of Tsigai sheep breed (from standards of commercial formula) can cause considerable deficiency in productivity of animals.

Keywords: Feeding, Live weight, Local breeds, Productivity, Wool quality.

INTRODUCTION

One of the most important tasks of the agro-industrial complex of Ukraine is the production of high-quality food products, in particular, meat and milk (multi-purpose animal breeding) (Chemerys et al., 2020). In this regard, the role of the sheep breeding industry, which produces milk, meat, and fat, is growing significantly (Peshko, 2022). Sheep breeding has no equal in terms of the variety and uniqueness of the products obtained from it and the ability to effectively produce (Kitayeva and Novichkova, 2023). The successful development of global sheep breeding and its competitiveness are largely determined by increased attention to meat and dairy productivity (Zygoyiannis, 2006; Pulina et al., 2018). With an almost constant level of wool production in the world over the past 12 years, the production of meat and sheep milk has increased by 70-80% (Ulyanov et al., 2011).

Currently, the economic value of various types of sheep products has changed in a positive direction (Duisebayeva, and Campbell, 2023; Bessell et al., 2023). In the fattening sheep industry, the focus is on the production of lambs, which account for more than 90% of the industry's total production value, of which up to 80% are lambs of the current year of birth (Erokhin et al., 2012).

The meat productivity of sheep is an integral indicator of signs caused by morphological, genetic, climatic and other factors and individual characteristics of animals (Tibbo, 2006). The main factor that determines the level of productivity of any breed of sheep is complete feeding, which leads to the receipt of high-quality products, better and more complete realization of genetically embedded high productivity and an increase in the period of economic use of animals. Improving the nutrition of sheep's diet by 30% and enriching it with mineral additives helps to increase the meat productivity of young sheep (Omarov, 2016).

At the current prices for wool and mutton and the actual costs of keeping animals, sheep breeding can be competitive due to increased fertility (Kosgey, 2004). Fertility is evidenced by the fact that different breeds have different fertility of ewes. But phenotypic factors also have a great influence on the fertility of sheep, as a result of which the

Citation: Kitaeva A, Mamedova V, Bezaltychna O, Slyusarenko I, and Novichkova A (2023). Productivity of the Tsigai sheep breed under different feeding regimens. *Online J. Anim. Feed Res.*, 13(6): 451-459. DOI: https://dx.doi.org/10.51227/ojafr.2023.62

coefficient of inheritance of fertility is very low, only 10-20%, since twin sheep more often give birth to twins compared to identical sheep, which characterizes the hereditary condition of multiple fertility (Zeitoun et al., 2020). Thus, in order to obtain a high commercial profit from sheep breeding, it is desirable to use large ewes in order to improve the reproduction of the herd (Hamilton and Hamilton, 2002). The high percentage of lamb mortality from birth to weaning leads to significant losses. There is a certain relationship between the live weight at the birth of lambs and their mortality. If at the birth of merino lambs with a live weight of less than 1.8 kg, 65% of the lambs mortality, then at the birth of 1.8 to 2.7 kg, about 3.5% die, and from 3.6 to 4.0 kg, only 4.0%. If the weight of lambs at birth in mothers of thin-wool breeds significantly exceeds 4.0 kg, then their mortality increases again (Filatov, 2016). The adaptation of their mothers (ewes) to the natural, climatic and geographical conditions of the breeding area is of great importance in the preservation of lambs (McManus et al., 2014; Zokabend Konig et al.., 2016). The main factor restraining the growth of animal productivity is the imbalance of their diets in terms of basic nutrients.

In practice, the quality of feeding is achieved by improving the quality of feed, improving the structure of rations and enriching them with complex protein and biologically active feed additives (Salem and Smith, 2008). In this regard, the use of non-traditional feed additives in feeding, which contain the main nutrients in an optimal ratio, deserves attention (Romero-Huelva et al., 2017; Chisoro et al., 2023; Dida et al., 2023).

Along with the need to optimize feeding, which involves providing the diet with a sufficient level of energy, protein, minerals, and vitamins, the negative impact of feeding poor-quality feed contaminated with xenobiotics is noted in animal husbandry practiceAt the same time, the processes of digestion and absorption of fats, proteins, carbohydrates, and vitamins are not disturbed, the regenerative potential of body tissues increases, and immunity is strengthened (McDowell, 2000).

In this regard, polyunsaturated fatty acids (PUFA) content is higher in pasture-fed lambs supplemented with green ryegrass compared to lambs fed soybean meal. Feeding weaned Awassi lambs with hydroponic barley for 3 months showed a positive effect on feed intake, body weight gain, absolute and average daily live weight gain, and feed conversion compared to lambs that received a diet without hydroponic barley. The introduction of alfalfa hay into the diet of lambs at the rate of 300 g per head per day not only increases growth indicators, but also improves the physical parameters of the carcass and the quality of lamb meat (Ibrahim et al., 2016).

Tsigai sheep is a Slovakian-Hungarian milk-meat-wool breed of sheep (Krupova et al., 2009; Kusza et al., 2010). Tsigai sheep mature early, graze well and are fattened. This breed is well adapted to year-round grazing and to extreme climatic conditions (mountains, frost), and is also capable of running hundreds or thousands of kilometers. In order to increase the meat productivity of young sheep of the Tsigai breed, it is very important to study its reaction to different levels of feeding during ontogenesis (Angelow et al., 2011).

Due to the fact that animals of different breeds react differently to these conditions, the study of the influence of different levels of feeding on the development of productive traits in the Tsigai breed in the conditions of the southern steppe of Ukraine is relevant and important for increasing the efficiency of lamb and young lamb production and needs research in this direction.

The purpose of the research is to study the growth of live weight and indicators of wool productivity of the Tsigai breed under different levels of feeding at the early stage of ontogenesis.

MATERIALS AND METHODS

The research was conducted on a private farm the "Rozdilnianske" sewage treatment plant of the Rozdilnian district of the Odesa region of Ukraine, and it was in according to ethical regulation of “Odesa State Agrarian University” for animal behavior.

Two groups of ewes of the Tsigai breed of the first breeding class, 3.5-4 years old, 40 heads in each, were formed. Groups were formed according to the principle of analogues, taking into account productivity, class, body weight, the nature of the wool cover, wool shearing and exterior and constitutional features.

The feed of the experimental and control groups differed in total nutrition, digestible protein and mineral substances. The control group of ewes received a balanced diet in accordance with the standard of feeding of ewes, which contained 1.35 feed units and 135 g of digestible protein, 15 MJ of exchangeable energy. The experimental group of ewes received a ration that was below the norm in terms of total nutrition by 11.5%, in digestible protein by 27.5%. In the period from 20 days of age to weaning at 4 months of age, lambs obtained from ewes of the control and experimental groups were fed with flattened oats in the amount of 50 to 100 g per head/day, depending on age. Feeding with table salt and chalk at will, as well as hay of good quality.

The lambs obtained from the ewes of the experimental and control groups were raised in the same conditions of keeping by the pen-based method before weaning, and after weaning, they were grazed on natural pastures and fields after the harvest of grain crops in the summer, and in the winter - by the stall method with provision of ration feeding, balanced in total nutrition, digestible protein and minerals. It contained: 1.12 feed units, 115 g of digestible protein, 12.0 MJ of exchangeable energy. After weaning and up to 14 months of age, lambs born from ewes in the control group were additionally fed concentrates to the main diet at the rate of 200 g of crushed barley per head/day. Lambs born from ewes in the experimental group were not given concentrates. During the research, the growth and development of the offspring of ewes was studied by determining the variability of their live weight, qualitative and quantitative indicators of the wool

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cover, namely: length, thickness, strength, shearing of wool in physical mass and washed fiber, and the yield of washed fiber. Research was conducted according to generally accepted methods. The digital material was processed by the method of variational statistics according to Plokhinsky (1969) using a computer.

Statistical analysis

The main task that the researcher solves using the methods of biological statistics is to draw conclusions about the properties of the general discovery based on the study of the selective discovery. Basic formulas were the power and structural averages (Efimova, 2015). Power compounds were mixed based on the general formula: M = [∑ Xi k n] 1⁄k or $M = \sqrt{\sum}$ Xi k, Where M is the average value, xi – contacts (date), n – sample size, k – type comparisons, k = 1 – arithmetic mean, k = 2 – arithmetic mean.

The coefficient of variation (CV) is an indicator of the degree of variability of a characteristic, used to compare the characteristics of variability in different variation series: $CV = \sigma X \cdot 100$ %, where the standard deviation (σ) serves as the main indicator diversity of a trait in a group.

Method of analysis

1. Find the arithmetic mean value of the characteristic in the group using the formula: $x = (x_1 + x_2 + \ldots + x_n)/n = \sum x_1/n$ where ∑ is sum sign, x is value option and n is number of animals.
2. Find the standard deviation in this group using the formula: $\sigma = \sqrt{\frac{\Sigma(x_1 - \bar{x})^2}{n}}$
3. Find the coefficient of variation using the formula: CV= (SD/xbar) * 100.
4. Find the error of the arithmetic mean: Sx-m (we indicate in tables Sx): $m = \pm \frac{\sigma}{\sqrt{n}}$

Reliability of sample data

To determine the reliability of sample data, it is necessary determine three statistical quantities: statistical error (m), reliability criterion (t) and probability (P). I determined the confidence level (P) from the table

Student (T Student test)

Table with critical values of Student's t-Distribution shows confidence levels of values probability (P) and at the same time critical values of significance levels (p), therefore, the value of the confidence probability 0.95 (95%) corresponds to the critical value of the significance level.

0.05 (5% X respectively P0.99 (99%) = p0.01 (1%); P0.999 (99.9%) = p0.001 (0.1%)

Reliability scale according to Plokhinsky: not reliability NS: P>0.05, reliable**: P<0.01, high reliability, ***: P<0.001.

RESULTS AND DISCUSSION

In order to achieve the set goal, it was planned to solve the following tasks of A) investigating the completeness and balance of rations for ewes and lambs obtained from them in the period of ontogenesis after weaning; B) determining the conditions for keeping newborn lambs before and after weaning from their mothers; C) determining the live weight of newborn lambs and the dynamics of its variability in the process of ontogenesis up to 14 months of age; D) carrying out calculations of the absolute increase in the live mass of the young at all stages of ontogenesis from birth to 14 months of age; E) evaluating the indicators of wool productivity of lambs at different levels of feeding; and F) establishing the effect of more than the normalized feeding of lambs on the formation and development of productive traits.

When studying the growth and development of lambs of the Tsigai breed from birth to 14 months of age, which received different levels of feeding both in the intrauterine and post-uterine periods of growth, it was established that their growth and development is significantly influenced by full feeding. Thus, in the ewes of the control group, which received a complete balanced diet during the period of contraction, the lambs received a sufficient amount of nutrients during the period of intrauterine development, as a result of which they developed significantly better than the lambs of the experimental group of ewes, which did not receive a sufficient amount of nutrients in an unbalanced diet. The live weight of lambs at birth testifies to the effect of different levels of feeding of ewes on the intrauterine development of the fetus (Table 1). Those lambs that received complete feeding during the early period of ontogenesis achieved live weight indicators for the first class by 10 months of age, and those that did not receive complete feeding did not reach this indicator even at 14 months of age. Their live weight was 1.95 kg, or 5.7% less than the requirements of the first class when scoring the young of the Tsigai breed. The live weight of lambs obtained from ewes of the control group was greater than that of the lambs of the same age from mothers of the experimental group from 0.5 kg or 18.2% at birth to 16.7 kg or 52.2% at 14 months of age). Lambs obtained from control ewes also had higher growth intensity (Table 2).

Therefore, lambs that received complete feeding during ontogenesis had a significant advantage in absolute live weight gain compared to lambs that were raised at a low level of feeding. During the period from birth to 4 months of age, the lambs obtained from ewes of the control group exceeded the lambs obtained from ewes of the experimental group by 14.13 kg or 2.2 times. However, in the future, lambs obtained from ewes of the experimental group intensively

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gained growth speed, and their absolute increase in live weight from 4 to 6 months of age was greater by 2.38 kg or 67.0% than that of animals obtained from ewes. control group.

Table 1 - Age-related variability of live weight of lambs, kg

Lambs received from mothers / Age (month)	Control group			Research group		
	N	X±Sx	CV, %	N	X±Sx	CV,%
At birth	20	3.25±0.090***	13.9	20	2.75±0.105	16.6
1	20	9.31±0.290***	13.3	20	6.19±0.211	14.9
2	20	15.57±0.446***	2.5	20	8.78±0.294	14.6
4	19	28.60±0.605***	14.7	18	13.97±0.748	23.3
6	19	28.15±0.647***	13.6	18	19.90±0.888	19.4
8	19	29.80±0.933***	13.6	18	23.05±0.723	13.7
10	19	36.20±1.166***	14.0	18	25.35±0.519	8.9
12	19	39.80±1.176***	12.8	18	27.15±0.454	7.3
14	19	48.80±1.208***	11.2	18	32.05±0.573	7.8

Not significant (NS): P>0.05, **: P<0.01, ***: P<0.001

Table 2 - Differences in the live weight of lambs (kg)

Lambs group / Growth period (month)	Control group		Research group	
	N	X±Sx	N	X±Sx
0 - 4	20	25.35±0.347***	20	11.22±0.426
4 - 6	19	3.55±0.626	18	5.93±0.818*
6 - 8	19	2.65±0.790	18	3.15±0.808
8 - 10	19	6 .30±1.049**	18	2.30±0.621
10 - 12	19	3.70±1.171	18	1.80±0.486
12 - 14	19	8.90±1.192**	18	4.90±0.513
0 - 14	19	45.55±0.649***	18	29.30±0.339

Not significant (NS): P>0.05, **: P<0.01, ***: P<0.001

Table 3 - Age-related dynamics of the length of the lambs depending on the level of feeding.

Lambs group / Age (month)	Control group			Research group		
	N	X±Sx	CV, %	N	X±Sx	CV, %
At birth	20	0.4±0.020	20.5	20	0.4±0.021	25.6
1	20	1.1±0.064	24.7	20	1.1±0.108	44.0
2	20	2.5±0.077***	13.1	20	1.9±0.145	32.6
4	19	3.8±0.076**	18.8	18	3.4±0.097	12.4
6	19	4.9±0.307NS	27.6	18	4.0±0.246	26.8
8	19	5.7±0.341NS	25.9	18	4.7±0.224	20.8
12	19	7.5±0.345***	20.2	18	5.8±0.209	15.5
14	19	9.8±0.360NS	10.1	18	8.8±0.150	17.4

Not significant (NS): P>0.05, **: P<0.01, ***: P<0.001

The period from 4 to 8 months of age is stressful for lambs. During this period, they stop receiving mother's milk and switch to a new type of feeding and independent living conditions, separated from their mothers. Their body adapts to new, unfamiliar conditions of keeping and feeding. Each animal reacts differently to such living conditions, which largely depends on the individual characteristics of the animals.

Ewes obtained from ewes of the control group, despite the fact that before weaning had higher indicators of live weight and absolute growth, after weaning felt the negative impact of stress more strongly and reacted to it by decreasing the rate of growth of live weight, which affected the indicators of its absolute increase. During the growth period from 8 to 10 months of age, lambs obtained from ewes of the control group prevailed over lambs obtained from ewes of the experimental group by 4.0 kg or 2.7 times in absolute live weight gain. This advantage was preserved during the early and subsequent periods of individual growth. During the period of growth from 12 to 14 months of age, their absolute growth increased compared to the bright ones obtained from ewes of the experimental group by 4.0 kg or by 81.6%, and during the entire period of rearing from birth by 14 months of age by 16.25 kg or by 55.4%.

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In small ruminant, in the process of individual development, with increasing age, the intensity of growth processes decreases, but this does not happen uniformly and periodically (Hoffman and Valencak, 2020). A period of temporary growth retardation is followed by a period of increased growth of tissues, organs and the entire body of the animal. Irregular growth with insufficient supply of nutrients to animals leads to uneven growth and development of various organs and tissues (Lawrence et al., 2012). This is due to the reproductive capacity of ewes, when during the period of confinement they must use part of the nutrients for the growth and development of their fetus, and after lambing - for the production of milk. With unsatisfactory feeding, they produce a smaller amount of products with worse technological qualities, in according to Blache et al. (2008) and Ochoa Cordero et al. (2019).

The wool productivity of sheep is affected by many factors, one of which is feed or dietary regimen (Hynd and Masters, 2002). Thus, present research has confirmed that the length of the wool in the Tsigai breeds depends on the level of providing them with nutrients and minerals. Brights obtained from ewes of the control group at all age periods of growth had longer wool than brights obtained from ewes of the experimental group (Table 3).

The given data are in the table 3 showed that up to one month of age, there were no differences in the length of the wool between the bright ones obtained from the mothers of the control and experimental groups. A statistically significant difference with varying degrees of probability was observed only after 2 months of age. A high degree of probability of the difference in wool length was found in lambs of the control group at 2- and 12-months of age and was 0.6 cm or 31.5% and 1.7 cm or 29.3%, respectively. In other age periods of growth of lambs, the difference in wool growth in length ranged from 0.4 to 1.0 cm or from 11.3 to 22.5% at .

Differences in the length of wool in the ewes obtained from ewes of both groups became significant after one month of age, when they began to receive supplementary feeding which is in agreement with Behrendt et al. (2011) in merino breed. This indicates that feed intake and nutrient absorption in ewes from control ewes were better than ewes from experimental ewes, and receiving additional nutrients with concentrated feed after weaning contributed to better growth wool in length. This is also due to the fact that the foals obtained from mothers who received balanced, complete nutrition during the gestation period had better conditions for development even in the fetal period and were born more viable and developed. Inadequate feeding also contributed to lower milk yield of ewes, as a result of which the development of their offspring was worse compared to the offspring of ewes that received complete feeding.

Production verification of the received data confirmed the results of our research. For this, two groups of lambs grown at different levels of feeding were individually evaluated. One group of lambs was grown in all periods of individual development on balanced rations, and the second received a ration below the norm in terms of total nutrition by 11.5%, and in terms of digestible protein by 27.5%. The results of the research showed that lambs, which received a ration below the norm, had shorter wool length compared to lambs, which received full feeding. Lambs that received a ration below the norm had a shorter length of wool by 1.7 cm or 22.67% compared to lambs that received full feeding. The lambs of wool in the length of the lambs of both groups, although there were significant differences, was not statistically significant. Taking into account the fact that wool longer than 5 cm is used for the production of worsted yarn, the length of wool of the evaluated sizes of both groups meets the requirements of the textile industry.

The level of animal feeding affects not only the growth of wool in length, but also the mass of wool and its quality indicators, that is, wool productivity (Table 4).

Table 4 - Qualitative indicators of wool of lambs

Lambs group / Indicators	Control group		Research group	
	X±Sx	CV, %	X±Sx	CV, %
Wool shearing, kg	7.5±0.345	20.2	5.8±0.209	15.5
Physical mass	3.60±0.108	13.3	2.84±0.198	29.1
Washing fiber	1.62±0.036	22.2	1.28±0.019	54.4
Strength, km (breaking length)	9.19±0.157	7.5	8.75±0.123	6.1

Table 5 - The thickness of the wool of lambs depending on the level of feeding

Wool thickness / The level of feeding		According to the norm		Below the norm	
Mkm	Quality	Heads	%	Heads	%
14.5 – 20.5	80 - 70	-	-	4	22.2
20.6 – 25.0	64 - 60	5	26.3	3	16.6
23.1 – 27.0	58 - 56	10	52.6	8	44.4
27.1 – 31.0	50 - 48	3	15.8	2	11.2
31.1 – 40.0	46 - 44	1	5.3	1	5.6
Total	-	19	100	18	100

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Table 6 - Shearing of wool of lambs depending on the level of feeding (kg)

Control group / The level of feeding	Physical mass	Washing fiber
According to the norm, (n = 200) Below the norm: (n = 225)	3.04±0.052	1.62±0.036***
Fodder units on 11.5% Digestive protein on 27.5%	2.73±0.112	1.28±0.019

Not significant (NS): P>0.05, **: P<0.01, ***: P<0.001

Table 7 - The thickness of the wool of lambs depending on the level of feeding

The level of feeding / The thickness of the wool		According to the norm (n=200)		Below the norm 11.5% for feeding unit 27.5% protein (n=225)	
Mkm	Quality	Heads	%	Heads	%
14.5 – 20.5	60	2	1.0	2	0.9
20.6 – 25.0	58	14	7.0	47	20.9
23.1 – 27.0	56	65	32.5	101	44.9
27.1 – 31.0	50	95	47.5	63	28.0
31.1 – 40.0	48	24	12.0	12	6.3
Total	-	200	100	225	100

Wool shearing in the physical lambs of the control group's lambs was 0.76 kg or 26.7% greater than that of the experimental group's lambs, after shearing wool in the washed fiber was 0.34 kg or 26.5%. Under optimal feeding conditions, lambs is a predictive factor of herd productivity. But providing sheep with fodder in sufficient quantity in accordance with their physiological state and feeding norms is, unfortunately, a problem, especially in commercial herds, where their diets are unbalanced in terms of nutrients and minerals, which negatively affects wool productivity. The technological properties of wool fibers largely depend on their tensile strength, which primarily determines the wearability, durability and duration of use of woolen products. The strength of wool in litters obtained from the mothers of the control group was higher than that of their peers from the mothers of the experimental group. The wool strength of the lambs of the control group is greater than that of the lambs of the experimental group by 0.44 km of breaking length or 5.0% than that of the lambs of the experimental group. In all age periods, the tensile strength of wool met the requirements of semi-fine wool. The technological properties of wool are also determined by its thickness. The thickness of wool is influenced by breed, age, individual characteristics, as well as the conditions of feeding and keeping sheep. The thickness of the wool of the furrows obtained from the ewes of the experimental group in the upper growth zone had a greater tendency to thin than the furrows obtained from the control group of ewes (Table 5).

Fluctuations in the thickness of the wool in the experimental group were in the range from 14.5 to 40.0 μm or from 80 to 44 quality, while in the animals of the control group, the fluctuations in the thickness of the wool were in a much smaller range (from 20.6 to 40.0 μm or from 60 to 46 qualities. The largest number of animals in the control group (14 heads or 73.7%) had the thickness of wool characteristic of sheep of the Tsigai breed, and among the animals of the experimental group, 11 heads or 61.2%, respectively. 64 qualities (20.6 - 23.0%) in the control group was only 5.3% (1 head), and in the experimental group (3 heads or 16.6%). In addition, among the pits of the experimental group there were 4 heads or 22. 2% of animals with a wool thickness of 14.5-20.5 microns or 70-80 qualities, while in the control group there were no animals with this wool thickness.

Greater thinning of the wool in the animals of the experimental group is caused by the fact that during the embryonic development period, when the laying and formation of hair follicles took place, as a result of unsatisfactory feeding of the mothers (McGrice, 2010; Scoobie et al., 2015), the formation of wool fibers was unsatisfactory due to insufficient supply of nutrients, and since lambs are born overgrown with length wool cover up to 1-1.5 cm, then, based on the patterns of wool growth, the upper zone of the wool feels the greatest negative impact of unsatisfactory and inadequate feeding of the mother during pregnancy, which affected the thickness of the wool of the fetus by thinning wool fibers. This finding is in agreement with Scobie et al. (2015).

In the postnatal period of lamb development, their mothers (ewes) were provided with the same complete and balanced diet, and the lambs themselves, in addition to mother's milk, were fed with concentrates, which contributed to better wool growth in thickness. Therefore, providing animals with a sufficient amount of nutrients contributes to a better supply of them to the hair follicles, as a result of which the genetically determined thickness of the wool characteristic of Tsigai sheep is manifested. Thus, when good feeding conditions are created, the difference in wool thickness is leveled.

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However, the tendency to produce finer wool fibers still persists in previously underfed animals. The results of the production inspection of our research on the influence of different levels of maternal feeding on the wool productivity of the offspring, which was carried out on a large herd, are shown in Tables 6 and 7. Wool shearing in the physical mass of lambs that received complete feeding was greater by 1.42 kg (87.6%), and in washed fiber by 1.45 kg or 2.1 times compared to bright ones grown on a low level of nutrition.

The obtained data indicate that the normalized feeding of lambs contributed to the growth of a woolen coat with a thickness characteristic of sheep of the Tsigai breed. Thus, the largest number of lambs (184 head or 92%), whose feeding was carried out according to the norm, had a wool thickness of 48-56 qualities. Only 176 heads, or 78.2%, had this thickness of wool in the group of lambs that were grown on an inferior diet. In addition, in this group of animals, 49 heads or 2.2% had a thickness of wool of qualities 58-60, which is not typical for Tsigai sheep, which indicates a thinning of the wool fibers. Therefore, high productivity of sheep is possible only if the animals are provided with full nutrition.

CONCLUSION

Full-fledged feeding of ewes of the Tsigai breed ensures good development of offspring at all stages of ontogenesis and contributes to the birth of healthy, viable lambs that are capable of high productivity. Complete feeding of ewes could have helped to increase the live weight of offspring at birth: lambs by 0.5 kg or 18.2%, and rams by 0.45 kg or 12.9%. Feeding 200 g of crushed barley in addition to the diet contributes to an increase in the live weight of lambs at the age of 14 months by 16.7 kg or 52.2%, the absolute increase in live weight by 16.25 kg (55.4%). Balanced feeding of lambs could have helped to increase the length of wool at the age of 12 months by 1.7 cm (29.3%), shearing of washed wool by 0.34 kg (26.5%), and strength wool by 0.44 km of breaking length (5%).

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Vira Mamedova, postgraduate, ORCID: https://orcid. org/ 0000-0002-9053-6743; E-mail: mamedova_vera@ukr.net

Data availability

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

Authors' contribution

All authors have seen and confirmed the authenticity of all the raw data and contributed equally to the details of this manuscript.

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Competing interests

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

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MORPHOLOGY AND REPRODUCTIVITY PROFILING OF MALE SENDURO GOATS BASED ON AGE DIFFERENCES

Nur DUCHA[1], Lisa LISDIANA[1], Guntur TRIMULYONO, Fitriari Izzatunnisa MUHAIMIN[1], Nur Nadiah Md YUSOF[2], Nurdiana SAMSULRIZAL[2], Razif DASIMAN[3], Ahmad FUDHAILI[1], Giyanita Rahma Ayu PRAMESTI[1], and Johana Dian RAHAYU[1]

[1]*Faculty of Mathematics and Natural Sciences, State University of Surabaya, Ketintang; Surabaya, Indonesia*
[2]*Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam; Selangor, Malaysia*
[3]*Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam; Selangor, Malaysia*

Email: nurducha@unesa.ac.id

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ABSTRACT: Senduro goats, a local breed of meat and dairy goats from Indonesia, are recognized for their significance in improving goat breeding and preserving valuable genetic resources. However, limited information exists regarding the reproductive physiology of Senduro goats, which poses challenges to the development of breeding programs and the preservation of genetic resources. This study aimed to investigate the morphological and reproductive profiles of male Senduro goats at different ages, focusing on morphological characteristics, mating behavior, and sperm quality. Morphological characteristics are assessed through body length measurements, while mating behavior serves as an indicator of reproductive behavior. Macroscopic evaluations of sperm quality include assessments of color, viscosity, pH, and volume, while microscopic examinations encompass motility (mass and individual), viability, and spermatozoa membrane integrity. The results showed morphological similarities between juvenile and adult samples, with their testicular size being the only significant difference. Based on macroscopic and microscopic examinations, no significant differences were found between groups. From the results it was concluded that there were no distinct differences in morphological characteristics, mating behavior, and sperm quality between male Senduro goats in the juvenile and adult stages.

Keywords: Biometric assessment, Mating behavior, Morphology profiles, Semen quality, Senduro goat.

INTRODUCTION

Goats are highly adaptable livestock species thriving in tropical regions (Knight and Garcia, 1997). In general term, among the goat breeds, Senduro goats hold a significant position as a local Indonesian breed (Hariyono and Endrawati, 2023). This particular local breed emerged from successful crossbreeding between *Jamunapari* goats from Etawah, India and Indonesian Bean goats. As part of preserving the indigenous genetic material and mitigating the risk of extinction, it is crucial to continually increase the population of Senduro goats. Several methods, including natural and artificial mating, can be used to bolster Senduro goat populations.

For successful breeding programs, it is imperative to gather information on the morphological and behavioral characteristics of male Senduro goats (Birhanie et al., 2019). Understanding reproductive behavior not only contributes to effective breeding but also aids in the evaluation of animal welfare (Zamiri et al., 2010). Additionally, a comprehensive understanding of the morphological features and behavioral patterns of male goats is crucial for breeding and cultivation programs, as these factors can be influenced by environmental conditions and the age of the animal (Dias et al., 2017). Behavior and morphology are often associated with the age of goats (Ambali et al., 2018). Studies on Sokoto Goats have shown differences in morphological characteristics and sperm quality with increasing age, where older goats tend to exhibit higher rates of sperm abnormalities (Akpa, et al., 2013). Evaluation of morphological traits is equally essential, as it shows a correlation between morphological characteristics and age in Boer goats (Abd-Allah et al., 2019). The age factor also plays a role in the production and reproduction capabilities of Senduro goats (Khandoker et al., 2018).

The reproductive performance of goats is influenced by age, nutrition, and seasonal factors (Đuričić et al., 2021; Ali et al., 2022). This kind of performance is closely associated with the apparent morphological characteristics. A previous study fconducted by Soutu et al. (2017) showed that testicular measurements, including length, width, and volume, are correlated with the age of male goats. These parameters can serve as a criterion for selecting exceptional young males. Age and nutritional status have also been found to influence the reproductive characteristics of sheep (Ptáček et al.,

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2017). However, there is a paucity of reviews on the morphological and reproductive characteristics of Senduro goats, an indigenous breed in Indonesia.

Therefore, this study aims to observe male Senduro goats of various ages to investigate the relationship between age and morphological and reproductive characteristics.

MATERIALS AND METHODS

Ethical regulation

The implementation of this research previously received research ethics, namely 070-KEP-UB-2023.

Determination of animals testing groups

The study was conducted at the Senduro Goat Breeding Center in the Senduro district of the Lumajang Regency of East Java, Indonesia. Data were collected from a total of 18 male Senduro goats, which were divided into two groups, including juveniles (ages 8 months to 1.6 years) and adults (aged 2 to 4.25 years). Each group consisted of nine males.

Morphological observations

Morphological observations of the goats included height, body length, body circumference, face shape, body shape, leg shape, presence or absence of horns, tail shape, hair color, hair distribution, presence or absence of beard, beard length, testicular length, testicular width, testicular size, and reproductive behavior. The testicular size was determined by measuring the length and diameter of the testicle using tape (Varghese et al., 2019). Meanwhile, testicle length was measured from the caudal part to the attachment point at the end of the scrotum. The circumference of the scrotum was evaluated to determine the diameter of the testicle. Length-measuring instruments were used for the morphological observations.

Observation of reproductive behavior

Reproductive behavior was observed based on libido behavior during mating or when collecting sperm using artificial vaginal techniques. The observed reproductive behaviors included the time of erection, frequency of mounting for ejaculation, and ejaculation time.

Fresh semen collection

After stimulation with a doe, sperm was collected using an artificial vagina. A total of 18 sperm samples were successfully collected from each goat and immediately placed in a water bath at 37°C. The collected sperm samples were then subjected to macroscopic and microscopic examinations. Macroscopic observations included assessing volume, color, consistency, pH, and spermatozoa concentration. Meanwhile, the microscopic analysis involved evaluating spermatozoa motility, viability, and integrity of sperm membranes.

Macroscopic observation

About two different studies independently conducted observations of fresh sperm volume, color, and consistency. pH was determined by using pH paper (Sigma-Aldrich), whereas spermatozoa concentration was determined using a counting chamber method. To perform the analysis, semen was diluted with Natrium Chloride (NaCl) solution, added to a haemocytometer, and observed under a light microscope at 400x magnification (Olympus).

Microscopic observation

Observations of spermatozoa motility

Both mass and individual motility of spermatozoa were assessed (Hayati et al., 2019). For mass motility evaluation, 20 µL of fresh sperm was added to a glass slide and observed under a light microscope (Olympus) at 40x magnification. Individual motility observations were performed by placing 10 µL of sperm on a glass object, followed by examination under a microscope at 400x magnification.

Observations of Spermatozoa Viability

Viability observations were conducted using Eosin-negrosin staining (Merck, Germany). About 10 microliters (µL) of sperm was placed on a glass object, followed by the addition of 20 µL of Eosin-nigrosin dye. The mixture was homogenized and allowed to rest for 30 seconds (Kamal et al., 2022). Subsequently, the sample-dye mixture was added to the glass slide by gently pushing it through the edge of the glass using another glass object at a 45° angle. The sample was allowed to dry at room temperature. After drying, observations were made under a light microscope at 400x magnification. The viability of spermatozoa was then determined by counting 200 cells in one field of view. Dead spermatozoa were stained purple, while live spermatozoa remained colorless (Srivastava et al., 2017; Ducha et al., 2020).

Observations of Spermatozoa Integrity

Spermatozoa membrane integrity was evaluated using the Hypo Osmotic Swallen Test (HOST). The HOST solution consisted of 0.9 g of fructose (Merck, Germany) and 0.49 g of sodium citrate fructose (Merck, Germany) dissolved in 100 ml of distilled water (Khan et al., 2017). About 100 microliters (µl) of fresh sperm was added to 1 ml of the HOST solution and incubated for 60 minutes at 37°C. To assess sperm swelling, 15 µl of the well-mixed sample was placed on a

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warmed slide (37°C), covered with a coverslip, and observed under a light microscope at 40x magnification. Swollen sperm cells with intact membranes were considered normal and indicative of fertilization potential. About 300 sperm were counted per slide, and the percentage of swollen sperm was calculated (Jamali et al., 2019).

Data analysis

Most of the obtained data were analyzed descriptively, including calculating averages, sums, and standard deviations and analyzing qualitative data. Quantitative data from macroscopic and microscopic observations of fresh sperm were further analyzed using the Mann-Whitney U Test (IBM SPSS 23) to determine statistical differences between the juvenile and adult age groups.

RESULTS AND DISCUSSION

Male Senduro goats generally exhibit a straight, convex face shape, white hair, a beard, and horns (in some males). Long hair is predominantly observed on the head, neck, chest, front and hind legs, beard, buttocks, and a straight and short tail (Table 1 and Figure 1).

These results align with the previous observations made by Ciptadi et al. (2019), who reported similar morphological characteristics in male Senduro goats, including a straight body with a convex face shape, white hair, a beard, and the presence of horns in most males. Additionally, dominant long hair was noted on the head, neck, chest, front and hind legs, beard, buttocks, and a straight and short tail (Ciptadi et al., 2019). Senduro goats share morphological similarities with the Etawah Peranakan goats. As an Etawah crossbreed, Senduro goats exhibit a convex face, long ears, black and white hair, horns, and thicker and longer hair on the neck and legs (Susilorini et al., 2020). These similarities can be attributed to the fact that Senduro goats are the result of crossbreeding between Etawah Peranakan goats, Kacang goats, and Jawarandu goats (Susilorini et al., 2020). Based on SNI 2018 data, Senduro goats can be characterized by white fur, a convex face, downward-hanging ears, the presence or absence of horns, a long beard in males, and a lack of beard in females. Furthermore, males exhibit longer body hair on the neck and hips, with long body hairs being more prominent and short tails. Male Senduro goats also display a larger body size compared to their female counterparts (Figure 2). Importantly, there were no significant morphological differences observed between adolescent and adult age groups (Table 2). These results align with the study conducted by Abd-Allah et al. (2019), who reported no variations in the morphological characteristics of Boer goats across different ages (Salman et al., 2019). This is also in line with the research of Sesay et al. (2022), that there are no significant differences in the morphology of West African Dwarf goats at various ages. No significant difference was observed in the reproductive behavior between adults and juvenile Senduro goats (Table 3). The analysis using the Mann-Whitney test also showed no significant differences between young and juvenile goats, indicating that age does not affect reproductive behavior. These results are supported by the study conducted by Suyadi et al. (2021), which found no variation in reproductive behavior among male Boer goats aged between 11 and 25 months (Suyadi et al., 2021). Furthermore, these results align with the observation of Hafizuddin et al. (2021), which showed no variation in libixdo levels among Etawah crossbreeds, both in juveniles and adults, with an average libido level of 3 times (Hafizuddin et al., 2021). Observation of the reproductive profile and its relation to the age of livestock, is very important to determine the breeding strategy and management of goats in an effort to increase the expected population (Parvathi et al., 2020).

Table 1 - Morphology male Senduro goats between juvenile and adult

Parameters	Juvenile (8 Months - 1.6 Years)	Adults (2 - 4.25 Years)
Shape of face	Dominantly convex	Dominantly convex
The shape of the body	Dominantly proportionally straight	Dominantly proportionally straight
Shape of leg	Dominantly proportionally	Dominantly proportionally
The presence of a horn	The majority have a horn, but some have not	The majority do not have horns; some have
Shape of tail	Dominantly straight and short	Dominantly straight and short
Hair color	Dominantly white	Dominantly white
Hair distribution	Dominantly long in the head, neck, chest, front and back leg, beard, and buttocks	Dominantly long in the head, neck, chest, front and back leg, beard, and buttocks

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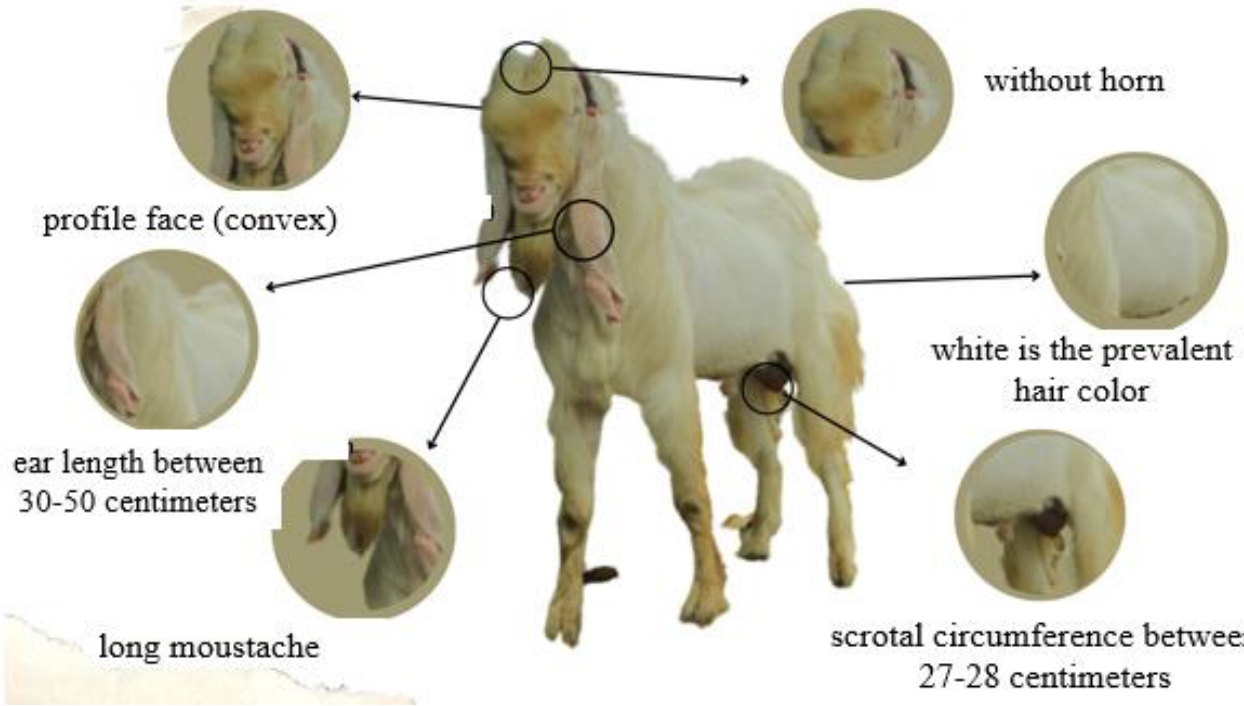

Figure 1 - Morphology character of male Senduro Goat

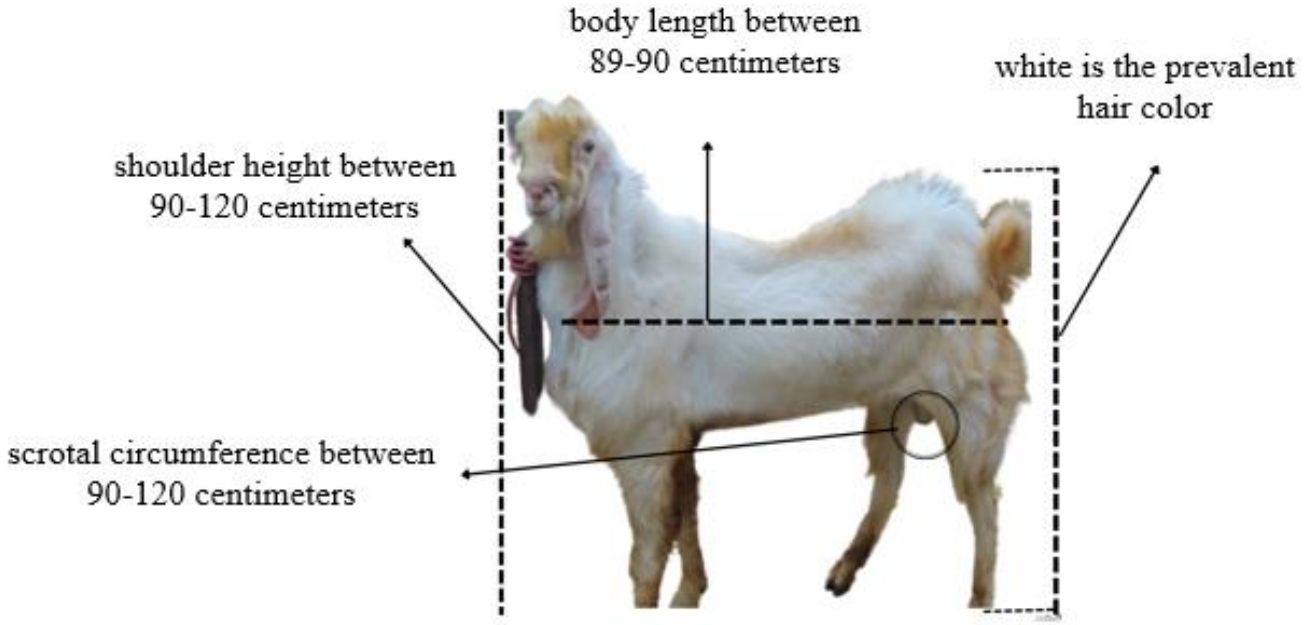

Figure 2 - Body posture of male Senduro goat

Table 2 - Statistical morphology between juvenile and adult Senduro goat

Parameters	Juvenile (8 Months - 1.6 Years)	Adults (2 - 4.25 Years)	Significant
Height (kg)	86.77±2.89^{bc}	97.22±2.43^{bc}	0.903
Body length (cm)	86±5.07^{b}	95.44±3.32^{b}	0.044
Length of beard (cm)	7.11±2.43^{bc}	15±2.42^{bc}	0.661
Circumference of the scrotum (cm)	26.11±0.99^{ab}	27.88±0.63^{ab}	0.052
Length of the scrotum (cm)	14.22±1.13^{a}	17.11±0.26^{a}	0.023
Chest size (cm)	90.22±2.28^{bc}	98.33±2.35^{bc}	0.521

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Table 3 - Reproductive behaviour of adult and juvenile Senduro goats

Parameters	Juvenile (8 Months - 1.6 Years)	Adults (2-4.25 Years)	Significance level
Erection Time (Seconds)	98.88±25.46	172.44±65.58	0.198
Number of false mountings: the frequency with which males do Mounting for ejaculation (Times)	2.55±0.44	2.8±0.38	0.495
Ejaculation time: the length of time it takes to ejaculate (times)	4.44±0.86	3.33±1.66	0.108

Table 4 - Macroscopic observations of juvenile and adult Senduro goats

Parameters	Juvenile (8 Months- 1.6 Years)	Adults (2-4.25 Years)	Significance level
pH	6.78±0.10	6.8±0.11	0.876
Volume (uL)	1000±172.40	911±107.29	0.109
Colour	Milky white	Milky white	-
Consistency	Thick	Thick	-
Smell	Fishy Odour	Fishy Odour	-

Table 5 – Microscopic observations of juvenile and adult Senduro goats

Parameters	Juvenile (8 Months- 1.6 Years)	Adults (2-4.25 Years)	Significance level
Mass motility	++++	++++	-
Individual motility	70.941±1.34[a]	72.246±2.07[a]	0.593
Viability	74.141±0.97[a]	76.480±2.583[a]	0.198
Membrane integrity	75.153±0.81[a]	77.341±2.84[a]	0.146
Concentration	1.26 x 10^8 [a]	1.27 x 10^8 [a]	0.197

Macroscopic and microscopic observations showed no significant differences. The macroscopic test involved evaluating volume, aroma, viscosity, color, and pH. Juvenile Senduro goats exhibited a pH value of 6.78±0.10, sperm volume of 1000±172.40 uL, milky white semen color, thick consistency, and a characteristic fishy odor of sperm (Table 4). Similarly, adult male goats displayed a pH value of 6.8±0.11 and sperm volume of 911±107.29 uL, along with milky white semen color, thick consistency, and a characteristic fishy odor of sperm. The macroscopic examination of spermatozoa showed no significant differences. Hafizuddin et al. (2021) reported that sperm quality in Anglo-Nubian goats, Etawah goats, and Ampera goats was not significantly affected by age or social interaction (Hafizuddin et al., 2021). In addition to macroscopic observations, microscopic evaluations of the sperm of Senduro goats were performed.

Subsequently, the microscopic evaluation was conducted to assess sperm quality based on parameters such as motility (movement), viability, and membrane integrity. The results showed that fresh semen from both juvenile and adult Senduro goats exhibited mass motility of ++++, indicating highly progressive and concentrated movement of spermatozoa colonies (Table 5). The average percentage of individual motility ± standard deviation (SD) in juvenile groups was 70.941±1.34, while adult goats had an average individual motility of 72.246±2.07. The analysis results showed that male age had no significant effect on the motility of fresh semen in both juvenile and adult groups. However, the average motility of individual spermatozoa was higher in adult goats. These values fell within the normal range, as reported by Syarifuddin et al. (2022), who indicated that the motility of fresh semen of Etawah ranged from 70-75% (Syarifuddin et al., 2022). The best viability of fresh semen was observed in the male group aged 2 - 4.25 years, with an average viability of 74.141±0.97, while juvenile goats had an average viability of 76.480±2.583. Similarly, the best spermatozoa membrane integrity was observed in the male group aged 2 - 4.25 years, with an average of 77.341±2.84, whereas juvenile goats exhibited membrane integrity of 75.153±0.81. The concentration of spermatozoa in Senduro goats showed no significant difference between the juvenile and adult groups, with an average concentration of 1.26 x 10^8 in juvenile goats and 1.27 x 10^8 in adult goats. These results are consistent with the study of Souto et al., 2017, which also reported no significant difference in spermatozoa concentrations (Souto et al., 2017). Although the sperm quality was evident, it was found to be higher at a more mature age. This is in line with the results of Lacuesta et al. (2015), who observed an increase in spermatozoa quality with age until adulthood (Lacuesta et al., 2015). Furthermore, Nishimura et al. (2000) showed an improvement in sperm quality as Tokara goats transitioned from juvenile to adult age.

CONCLUSION

In conclusion, the results of this study showed that there were no significant differences in terms of morphology, reproductive behavior, and sperm quality between adult and juvenile male Senduro goats. However, the data suggested that the age range of 2 to 3.5 years was optimal for producing spermatozoa with good quality. Based on the results of

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this research, it can be used as a guideline in selecting male Senduro goats that are ready to mate based on morphological and reproductive characteristics.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Nur DUCHA; E-mail: nurducha@unesa.ac.id

Data availability

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

Authors' contribution

All authors played a role in the research and writing of this article. N. Ducha, N.N. Yusof, N. Samsulrizal, E.R. Damiman played a role in designing the research. The research carried out in the field and laboratory was carried out by N. Ducha, L. Lisdiana, G. Trimulyono, F.I. Muhaimin, A. Fudhaili, G.R.A. Pramesti., J.D. Rahayu. Data analysis was carried out by F.I.Muhaimin, A. Fudhaili, N.N.Yusof, N.Samsulrizal, E.R.Dasiman, G.R.A. Article writing was carried out by all authors.

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Consent to publish

The authors agree to the publication of this manuscript.

Competing interests

The authors declare no competing interest

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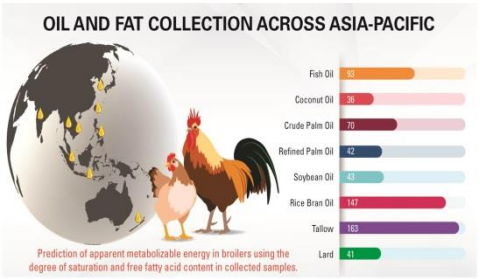

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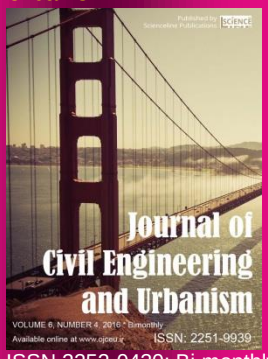

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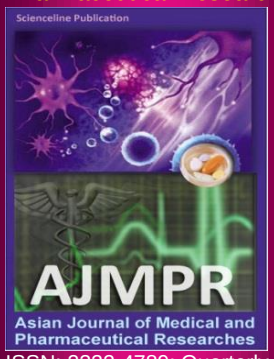

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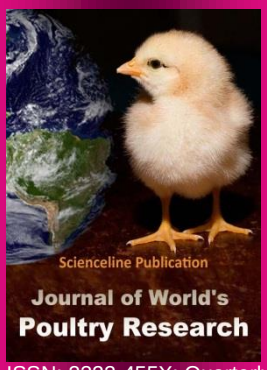

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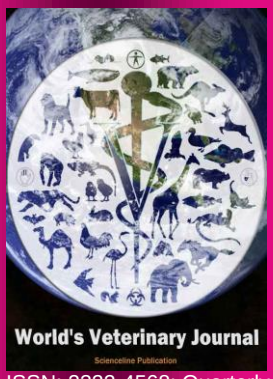

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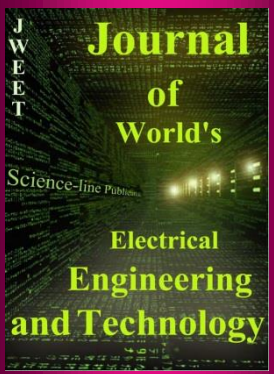

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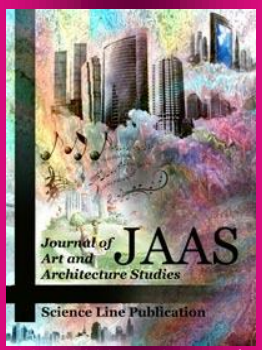

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