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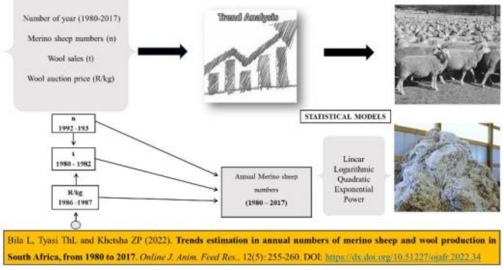
Volume 12 (5); September 30, 2022

Research Paper

Trends estimation in annual numbers of merino sheep and wool production in South Africa, from 1980 to 2017



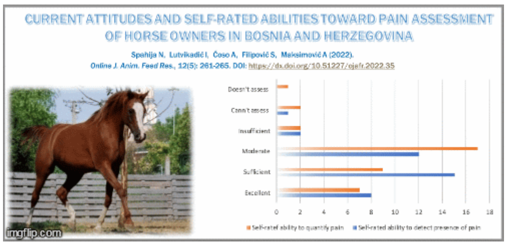
Abstract
The economic important value of wool in South Africa is influenced by many characteristics that meet processor capacities and consumers' preference. The study was conducted to characterize the trends of annual Merino sheep numbers, wool sales and wool auction price in South Africa, from 1980 to 2017. The data from year 1980 to 2017 was retrieved from the abstract of agricultural statistics which was published by the Department of Agriculture, Land Reform and Rural Development (DLRRD) in 2020 was used. Trends were analysed using the differences and percentage change between numbers of years in each independent variable (annual number of Merino sheep, wool sales and wool auction price). The findings indicated that there was a trend in annual number of Merino sheep, wool sales and wool auction prices. In annual numbers of Merino sheep, the highest decrease was observed from 1992 to 1993 (-1878) by 11.2 percent (%) while the huge increase was observed from 1999 to 2000 (+1177) by 10.6%. In wool sales, the findings showed that there was an increase (+5.4) from 1980 to 1982 with 7.7% while there was a decrease (-4.7) from 1982 to 1983 by 6.2%. In wool auction price, the highest increase was observed from 1986 to 1987 (+250903) by 77.2%. Regression findings indicated that all the predicted regression models were statistically significant at $P < 0.01$. However, the results suggest that quadratic ($R2 = 90$) was the best fit model to predict the relationship between number of years and annual Merino sheep numbers. This study suggests that there was a trend in annual numbers of Merino sheep, wool sales and wool auction prices in South Africa from 1980 and 2017. Moreover, this study might help Merino sheep farmers to recognise the trends that might be helpful in planning for annual wool productions.

Keywords: Exponential, Linear, Logarithmic, Quadratic, Sheep.

[Full text-PDF]

Short Communication

Current attitudes and self-rated abilities toward pain assessment of horse owners in Bosnia and Herzegovina



Abstract
Over the past decades, recognised importance of prompt and valid pain recognition and quantification in veterinary medicine significantly increased interest in investigating attitudes and self-rated abilities of veterinarians towards pain assessment. However, giving that the owners are the ones who decide when to call the veterinarian, it is also essential to investigate their attitudes and knowledge regarding pain recognition and management. This is the first research investigating horse owners' attitudes and self-rated abilities towards pain assessment and management in Bosnia and Herzegovina. The participants were invited via email or social media with attached link to online questionnaire created using the Google Forms platform. Surveyed population included adult sport or pleasure horse owners, and horse caretakers in Bosnia and Herzegovina. Study response rate was 33.33% (40 respondents). The questionnaire consisted of sections asking about respondents' demographic data, general pain assessment and management, and attitudes towards pain assessment done by veterinarians compared with theirs. Study respondents considered their abilities for pain recognition and quantification as sufficient and sufficient/moderate, even though most of them were unfamiliar with pain scales, and only negligible number use them. This study indicates the need and importance of owners' education concerning pain recognition and quantification in horses, and their more effective communication with veterinarians.

Keywords: Horse, Pain recognition, Pain quantification, Pain scales, Questionnaire.

[Full text-PDF]

Research Paper

Value-added analysis of the meat agroindustry in Indonesia

Sikone HY, Hartono B, Suyadi, Utami HD and Nugroho BA.

Online J. Anim. Feed Res., 12(5): 266-271, 2022; pii: S222877012200036-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.36

Sikone HY, Hartono B, Suyadi, Utami HD and Nugroho BA (2022). **Value-added analysis of the meat agroindustry in Indonesia.** *Online J. Anim. Feed Res.,* 12(5): 266-271. DOI: https://dx.doi.org/10.51227/ojafr.2022.36

Abstract
This study analyzes the added value of processing fresh beef into beef jerky, shredded beef, and se'i (smoked beef) products in Kefamenanu. The research was conducted in February and March 2021. The research method used was the survey method. Data was collected from cattle slaughterers (butchers) in abattoirs (n = 7), meat retailers (n = 13), and MSMEs in the meat processing industry (n = 15) and consumers (n = 90). The sample was selected through a purposive sampling method, with the criteria for selecting a sample of MSMEs in the processing industry as follows: 1) entrepreneurs have beef jerky, shredded beef, and se'i (smoked beef); 2) entrepreneurs who have sold their products in the past year and their three products are circulating in the market; 3) entrepreneurs produce these three products sustainably. The data was analyzed using descriptive statistics, and the added values of beef jerky, shredded, and se'i (smoked beef) products were calculated using the Hayami method. The results showed that each processing of one kilogram of fresh beef could produce 0.70 kg (shredded), 0.73 kg (jerky), and 0.68 kg (se'i). The added value obtained is USD 3,56 for shredded products, USD 4,03 for jerky products, and USD 2,91 for se'i products. The profit from shredded beef is USD 3,34, with beef jerky of USD 3,80 and se'i (smoked beef) of USD 2,64.
Keywords: Beef fresh, Beef product, Farm management, Meat processing, Value added.

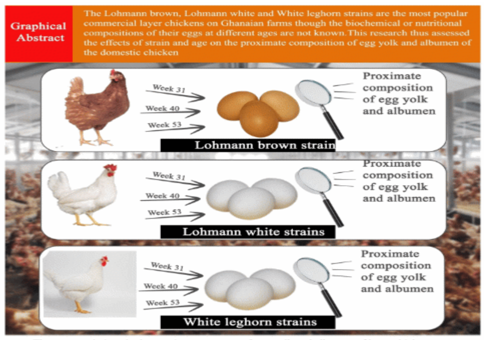
[Full text-PDF]

Research Paper

Effect of strain and age of layer chickens on proximate contents of egg yolk and albumen

Kruenti F, Hagan JK, Ofori SA, Lamptey VK and Adu S.

Online J. Anim. Feed Res., 12(5): 272-278, 2022; pii: S222877012200037-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.37



Abstract
This research assessed the effects of strain and age on the proximate compositions of egg yolk and albumen of the domestic chicken. A total of 504 eggs were used in a 3 X 3 factorial experiment involving the Lohmann white, Lohmann brown and White Leghorn which were 31, 40 and 53 weeks old using a completely randomised design (CRD). Data obtained were subjected to the two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure. Differences in means were separated using the Tukey pairwise comparisons method at 5% level of significance. The results show that, eggs from the Lohmann layers have significantly more protein but lower fat content in the yolk than the White leghorn; while albumen protein was slightly higher in the White leghorn with lower albumen fat in the white strains than the Lohmann brown. Protein content of egg yolk significantly increased as the birds advanced in age but albumen protein was not affected largely by layers' age. Yolk fat significantly decreased as the birds grew but albumen fat was not substantially affected by age of the hens. There was significant effect of strain by age interaction on yolk and albumen protein contents but not on their fat content across the chicken groups. There are variations in the proximate contents of egg yolk and albumen of layer chickens by virtue of their genetic constitution and ages; so, eggs must be produced from the best strains at the appropriate hen-ages to make their nutrient contents meet the needs of specific consumers and products.
Keywords: Genetic constitution, Lohmann white, Lohmann brown, Variations, White Leghorn.

[Full text-PDF]

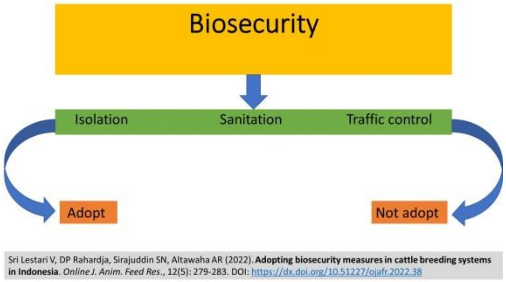
Research Paper

Adopting biosecurity measures in cattle breeding systems in Indonesia

Sri Lestari V, DP Rahardja, Sirajuddin SN, Altawaha AR.

Online J. Anim. Feed Res., 12(5): 279-283, 2022; pii: S222877012200038-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.38

Abstract
The study aimed to analyze the barriers to implementing biosecurity measures. Biosecurity refers to a collection of procedures designed to keep disease from spreading beyond the farm. Isolation, sanitation, and cage traffic control are all biosecurity strategies. At 2021, this study was carried out in Selli village, Bengo subdistrict, Bone regency, South Sulawesi province. Bone Regency is noted for being the largest livestock supplier in the province of South Sulawesi. A total of 35 people were chosen to be observed and questioned with the help of a questionnaire. The information gathered was analyzed using descriptive statistics. The results revealed that only 2.86 percent and 28.57 percent of respondents used quarantine or provided fences or barriers as isolation or segregation methods. Farm visitors were not provided with sanitary facilities. Everyone entering or exiting the cage was not subject to traffic control. The absence of information was the first roadblock to biosecurity adoption (65.71 percent). Then came a lack of time (31.43 percent) and a hefty price (2.86 percent). Therefore, training and extension should be provided by the Animal Hsubandary Services and Private Sectors.



Keywords: Adopting, Beef, Biosecurity measures, Cattle breeding, Smallholder farmers.

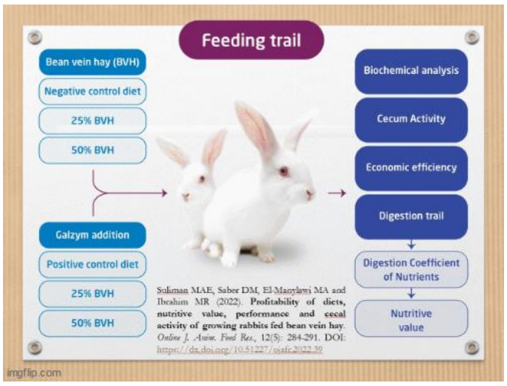
[Full text-PDF]

Research Paper

Profitability of diets, nutritive value, performance and cecal activity of growing rabbits fed bean vein hay

Suliman MAE, Saber DM, El-Manylawi MA and Ibrahim MR.

Online J. Anim. Feed Res., 12(5): 284-291, 2022; pii: S222877012200039-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.39



Abstract
A feeding trail was conducted to study effect of bean viens hay (BVH) on the growing rabbit's performance, nutritive value and cecum activity and economic return of experimental diets that. Seventy two New Zealand White (NZW) rabbits (6 week of age) were divided into 6 groups and were fed 6 experimental diets inclusion BVH and Galzym® enzyme contains cellulase, xylanase, lipase, amylase, protease, pectinase, arabinase, phytase, α-galactosidase, and β-glucosidase additives. Group 1 fed negative control (basal diet without both BVH and Galzym®) and group 2 fed positive control diets (basal diet without BVH and with Galzym®). The 3rd and 4th groups fed 25% BVH substitution of clover hay without Galzym® (T3) and with Galzym® (T4). The 5th and 6th group's rabbits fed 50% BVH substitution of clover hay without Galzym® (T5) and with Galzym® (T6). The crude fiber, NDF, ADF, ADL and cellulose were higher in BVH than those in clover hay while, CP% and digestible energy (Kcal/kg) were lower in BVH than those in clover hay. Results of interaction between BVH and Galzym® additives (treatment effect) had significant ($P<0.05$) effect on rabbits productive performance, all nutrients digestibility except EE and DCP%, blood biochemical (TP, albumin, globulin, albumin/globulin ratio, cholesterol, ALT, and urea), and cecum activity (TVFA's and NH3). Moreover, main effect of BVH was significantly ($P<0.05$) improved of rabbits productive performance, nutrients digestibility (DM, OM, CP, CF, and NFE%), blood constituents (TP, albumin, globulin, and ALT), and TVFA's in cecum. Enzyme main effect decreased ($P<0.05$) FI and formation of NH3 in rabbit's cecum. In conclusion, the dietary BVH improved the productive performance of growing rabbits without negative effect on health status. Enzyme addition increases the BVH utilization and diets profitability.
Keywords: Bean vine hay, Cecum activity, Cost, Digestibility, Rabbit.

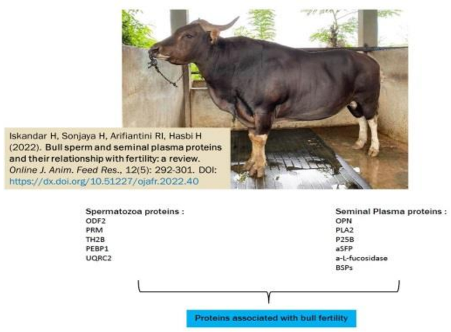
[Full text-PDF]

Review

Bull sperm and seminal plasma proteins and their relationship with fertility: a review

Iskandar H, Sonjaya H, Arifiantini RI and Hasbi H.

Online J. Anim. Feed Res., 12(5): 292-301, 2022; pii: S222877012200040-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.40



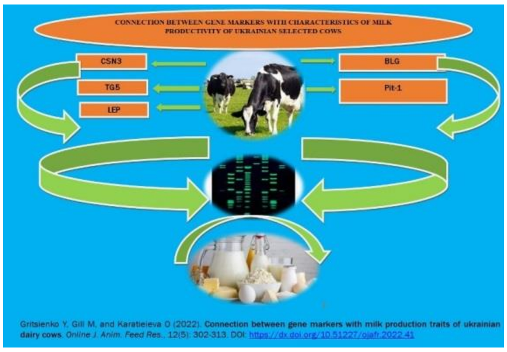
Abstract
The efficiency of artificial insemination (AI) is greatly influenced by the quality of semen. Spermatozoa and seminal plasma are found in semen, which play a role in the reproductive process and its ability to fertilize an egg and maintain the development of an embryo. Various factors will determine the fertility capacity of a sperm, both from the intrinsic factors of the sperm and the plasma component of the semen. Seminal plasma proteins are crucial for maintaining the stability of the membrane, viability, motility of spermatozoa, acrosome reactions,

maintaining osmotic pressure and helping the fertilization process. Good quality semen will support the fertilization process. The purpose of this scoping review is to increase our understanding of protein from sperm and seminal plasma of bulls and their relationship with fertility. The sperm proteins that were significantly correlated with fertility were Outer Dense Fiber protein 2 (ODF2), Protamine (PRM), Testis specific histine 2B (TH2B), Phosphatidylethanolamine binding protein (PEBP4), and Ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRSC2). Meanwhile, the seminal plasma proteins positively correlated with fertility were Osteopontin (OPN), Phospholipasea 2 (PLA2), P25b, Acidic seminal fluid proteins (aSFP), Alpha-L-fucosidase (a-L-fucosidase), and Binder of sperm (BSPs).
Keywords: Bovine, Fertility, Semen, Seminal plasma protein, Sperm protein.

[Full text-PDF]

Research Paper

Connection between gene markers with milk production traits of Ukrainian dairy cows



Gritsienko Y, Gill M, and Karatieieva O.

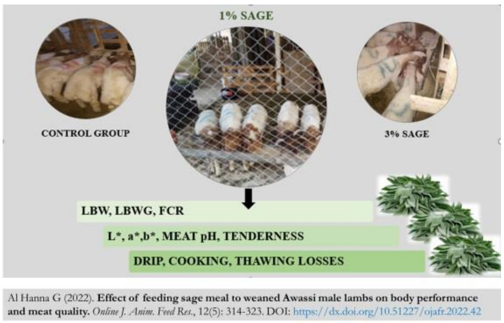
Online J. Anim. Feed Res., 12(5): 302-313, 2022; pii: S222877012200041-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.41

Abstract
The purpose of this study was to obtain information on the genotyping of cows of Ukrainian dairy breeds for the presented loci capa-casein gene (CSN3), thyroglobulin (TG-5) gene, leptin (LEP), pituitary-specific transcription factor (Pit-1), and beta-lactoglobulin (BLG) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and to test their association with milk production. The influence of the genotype on milk yield, fat and protein content in the milk of cows of Ukrainian selection has been established. The highest levels of milk productivity traits were expected from animals with the CT genotype for the LEP gene, AA for the CSN3 gene, CC for the TG5 gene, and AA and BB for the Pit-1 gene. But at the same time, milk obtained from cows with CSN3 BB genotype were characterized by the best cheese suitability. The effect of the homo- or heterozygous state of BLG on the signs of milk production was less noticeable, and the homozygous TT genotype for the TG5 gene was not found in any of the studied breeds. The results obtained can be used in the practical work of breeding farms along with traditional methods of selection, control and preservation of the genetic diversity of specific herds at an optimal level.
Keywords: Capa-casein, Marker genes, Polymorphism, Thyroglobulin, Leptin, Pituitary transcription Factor, Beta-lactoglobulin

[Full text-PDF]

Research Paper

Effect of feeding sage meal to weaned Awassi male lambs on body performance and meat quality



Al Hanna G.

Online J. Anim. Feed Res., 12(5): 314-323, 2022; pii: S222877012200042-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.42

Abstract
An experiment was conducted for 8 weeks on 15 Awassi lambs using sage dry meal. Animals were randomly allocated into 3 groups [CGM (0% sage; Salvia officinalis), EGM1 (1% sage) and EGM3 (3% sage)] by 5 heads. Feed intake at the 8th week increased proportionally with LBW. Cumulative live body weight gain increased slightly more in EGM3 after the 5th week to attain 10.7 Kg at 8th week. FCR was most effective and attained 3.83 ± 0.97 in EGM3 Vs 4.14 ± 0.53 and 4.15 ± 0.64 in CGM and EGM1, respectively. After cooling, luminance ranged between 45.41 ± 2.97 in CGM and 47.28 ± 5.63 in EGM1 where as in EGM3 it was 47.28 ± 5.63. Redness, a*, after cooling was lowest in EGM3 (20.15 ± 3.29) followed by CGM (22.61 ± 3.41) and EGM1 (24.97 ± 1.24). Yellowness b* after 1 month of freezing is positively correlated with the achieved results after 24 h of cooling. The least losses in water after cooling was in EGM3 attaining 11.39 ± 2.39%. Meat of CGM loses more water after cooking (30.30 ± 6.52%) than other groups. Furthermore, after 24 hours of cooling, the most tender meat was in EGM1 (4.87 ± 0.44mm) in comparison to CGM (3.3 ± 0.64mm), whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 ± 0.8mm). It is concluded and recommended to use rations containing sage meal in the daily feeding of Awassi sheep.
Keywords: Awassi lambs, Body performance, Feeding, Meat quality, Sage dry meal.

[Full text-PDF]

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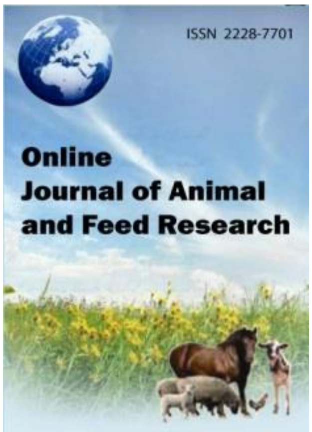

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DOI: https://dx.doi.org/10.51227/ojafr.2022.34

TRENDS ESTIMATION IN ANNUAL NUMBERS OF MERINO SHEEP AND WOOL PRODUCTION IN SOUTH AFRICA, FROM 1980 TO 2017

Lubabalo BILA[1], Thobela Louis TYASI[2] and Zenzile Peter KHETSHA[3]

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[2]*School of Agricultural & Environmental Sciences, Department of Agricultural Economics and Animal Production, University of Limpopo, Private Bag X1106, Sovenga 0727, Limpopo, South Africa*
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Supporting Information

RESEARCH ARTICLE
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ABSTRACT: The economic important value of wool in South Africa is influenced by many characteristics that meet processor capacities and consumers' preference. The study was conducted to characterize the trends of annual Merino sheep numbers, wool sales and wool auction price in South Africa, from 1980 to 2017. The data from year 1980 to 2017 was retrieved from the abstract of agricultural statistics which was published by the Department of Agriculture, Land Reform and Rural Development (DLRRD) in 2020 was used. Trends were analysed using the differences and percentage change between numbers of years in each independent variable (annual number of Merino sheep, wool sales and wool auction price). The findings indicated that there was a trend in annual number of Merino sheep, wool sales and wool auction prices. In annual numbers of Merino sheep, the highest decrease was observed from 1992 to 1993 (-1878) by 11.2 percent (%) while the huge increase was observed from 1999 to 2000 (+1177) by 10.6%. In wool sales, the findings showed that there was an increase (+5.4) from 1980 to 1982 with 7.7% while there was a decrease (-4.7) from 1982 to 1983 by 6.2%. In wool auction price, the highest increase was observed from 1986 to 1987 (+250903) by 77.2%. Regression findings indicated that all the predicted regression models were statistically significant at $P < 0.01$. However, the results suggest that quadratic ($R^2 = 90$) was the best fit model to predict the relationship between number of years and annual Merino sheep numbers. This study suggests that there was a trend in annual numbers of Merino sheep, wool sales and wool auction prices in South Africa from 1980 and 2017. Moreover, this study might help Merino sheep farmers to recognise the trends that might be helpful in planning for annual wool productions.

Keywords: Exponential, Linear, Logarithmic, Quadratic, Sheep.

INTRODUCTION

In South Africa the wool production industry continues to be an important fibre in textile trade market (Van der Merwe et al., 2020). The economic important value of wool in South Africa is influenced by many characteristics that meet processor capacities and consumers' preference (Van der Merwe et al., 2020). The quantities of wool production produced annually in South African market have declined from 1991 to 2005 (DAFF, 2018) this is resulted by increased stock theft cases and due to the meat prices that became the main source of income from sheep production. Moreover, this leads to increased crossbreeding developments with the use of terminal sires to improve growth and meat production of wool sheep breeds (Cloete et al., 2008). The wool production complements lamb production in the total income generated by farmers as when wool-type or dual-purpose dam lines are used in breeding programmes (Van der Merwe et al., 2020).

The difference in profitability of sheep breeds remains one of the most controversial discussion issues among farmers and is one of the aspects that receive little observation from researchers and scientists (Snyman et al., 2014). The reason for this is that the full implication of such a comparison is rarely taken into consideration (Snyman et al., 2014). However, farmers are continuously changing from one breed to another. The decision to change from one breed to another is mostly likely to be due to short-term financial reasons and current market trends favouring either wool or meat Snyman et al. (2014). However, to the greatest of our knowledge there is no literature documented about the trends of Merino sheep numbers and wool production in South Africa from 1980 to 2017. Hence the objective of this study was to estimate and describe the trends over time for annual Merino sheep numbers and wool production in South Africa from 1980 to 2017. This study might help Merino farmers and wool production industry of South Africa to recognize the outcomes of changes in number of years on annual Merino sheep numbers and wool production.

MATERIALS AND METHODS

Data collection

A thirty-eight years (1980 to 2017) data of annual numbers of Merino sheep and wool production from abstract of agricultural statistics (2020) was used in the study. The abstract of agricultural statistics which was compiled by

Citation: Bila L, Tyasi ThL and Khetsha ZP (2022). Trends estimation in annual numbers of merino sheep and wool production in South Africa, from 1980 to 2017. *Online J. Anim. Feed Res.*, 12(5): 255-260. DOI: https://dx.doi.org/10.51227/ojafr.2022.34

directorate statistics and economic analysis was published in the Republic of South Africa by Department of Agriculture, Land Reform and Rural Development. Data of annual numbers of Merino sheep was presented in thousands (1000) and wool production presented in thousand tons (1000 *t*) were used for the study.

Statistical analysis

Winston (2016) and Statistical Packages for Social Sciences version 26 (SPSS, 2019) were used for data analysis. The linear, logarithmic, quadratic, exponential and power regression analyses were used to estimate the rate of curves. The following mathematical model equations were used:

Linear: $y = \beta_0 + \beta_1 * x$

Logarithmic: $y = \beta_0 + \beta_1 * \ln(x)$

Quadratic: $y = \beta_0 + \beta_1 * x + \beta_2 * x^2$

Exponential: $y = \beta_0 + \beta 1 (\beta_{2x,1} + \beta_{p+1} x_{i,} 1) + e_{i6}$

Power: $y = \beta_0 * x^{\beta 1}$

The analysis of residuals was included to identify a typical dataset; these data were recognized by plotting the residuals against the values predicted by the equations. A typical data was eliminated if the corresponding values of the student residuals were outside the range of −2.5 to 2.5. The accuracy of the models was evaluated by the determination coefficients (r^2) and mean squared error (MSE) (SPSS, 2019). Lastly the goodness of fit criteria was performed to check the best model in this study. The following criterions were used:

$$R^2 = 1 - \left(\frac{SST}{SSE}\right) \quad ; \quad RMSE = \sqrt{\frac{SSE}{N - p - 1}} \quad ; \quad AIC = N \, Ln \left(\frac{SSE}{N}\right) + 2p \quad ; \quad BIC = N \, Ln \left(\frac{SSE}{N}\right) + p \, Ln \, N$$

Where, R^2 = coefficient of determination; SST = is the total sum of square; SSE = is the residual sum of square; RMSE = is the residual mean square error; N = is the number of observations; P = is the number of parameters in the regression equation; AIC = is the Akaike information criterion; BIC = is the Bayesian information criterion; Ln = is the natural logarithm in calculator.

RESULTS AND DSICUSSION

Descriptive statistics

The study was conducted to investigate the trends of annual Merino sheep numbers and wool production using different regression models (linear, logarithmic, quadratic, exponential and power). The descriptive statistics was computed to examine the summary of the dataset. Table 1 indicates the descriptive statistics of the data used in the study. The summary revealed that there were only thirty-eight observations (n = 38) on the annual Merino sheep numbers and wool production used for the study. Results further recognized that the annual Merino sheep numbers had the minimum number of 10466, maximum number of 20009, average of 13769, and standard deviation of 2909.12 while wool sales had a minimum number of 18.00. While revealing the maximum number of 76.30, average of 45.71 and standard deviation of 16.88. Our findings are in agreement with the reports of Ngambi and Belete (1999) that recognised that mohair production had a trend in Lesotho between 1935 and 1990.

Table 1 - Descriptive statistics of annual Merino sheep numbers and wool production

Statistics	N	Minimum	Maximum	Mean	Std. Deviation
Year	38	1980	2017	1998.50	11.11
Merino sheep numbers (n)	38	10466.00	20009.00	13769.58	2909.12
Wool sales (t)	38	18.00	76.30	45.71	16.88
Wool value (R)	38	161099.30	3408712.60	948974.92	836565.31
Auction price(R/Kg)	38	2.29	113.37	26.81	28.36

n: numbers; t: tons; R: rand; R/kg: rand per kilogram; kg: kilogram; Std. Deviation: Standard deviation.

Differences and percentage changes in annual Merino sheep numbers

In this study, annual number of Merino sheep differences and percentage changes were also computed from 1980 to 2017. The results of annual Merino sheep numbers (Table 2), differences and percentage change were calculated from 1980 to 2017. The results of annual Merino sheep numbers indicated that there were trends between years from 1980 to 2017 in the number of Merino sheep in South Africa. The highest decrease was observed from 1992 to 1983 (-1878) by 11.2% while the huge increase was observed from 1999 to 2000 (+1177) by 10.6%.

Differences and percentage changes in annual wool sales production

Results of differences and percentages changes in annual Merino sheep wool sales in South Africa from 1980 to 2017 are shown in Table 3. The findings showed that there was an increase (+5.4) in annual Merino sheep wool sales from 1980 to 1982 with 7.7% while there was a decrease (-4.7) from 1982 to 1983 by 6.2 %.

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Differences and percentage changes in annual wool value

Results of annual wool value production (Table 4) indicated that there were trends between years from 1980 to 2017 in the annual wool value in South Africa. The highest decrease was observed from 2002 to 2003 (-299702.3) by 28.0% while the huge increase was observed from 1986 to 1987 (+250903) by 77.2%.

Differences and percentage changes in wool auction price

The results of annual auction price (Table 5) indicated that there were trends between years from 1980 to 2017. The highest increase was observed from the year 2000 to 2001 (+8.5) by 55.0%, while the highest decrease was noticed from the year 1985 to 1986 (-1.8) by 35.3%.

Table 2 - Annual Merino sheep numbers and percentage change from one year to the next

Year	Annual number of Merino sheep (n)	Difference	Trend	Percentage Change
1980	20009			
1981	19335	-674		3.4
1982	19036	-299		1.6
1983	17660	-1376		7.2
1984	16551	-1109		6.3
1985	16045	-506		3.0
1986	16353	308		1.9
1987	16087	-266		1.6
1988	16161	74		0.5
1989	17644	1483		9.12
1990	17916	272		1.5
1991	17057	-859		4.8
1992	16762	-295		1.7
1993	14884	-1878		11.2
1994	14470	-414		2.8
1995	13331	-1139		7.9
1996	12862	-469		3.5
1997	12185	-677		5.3
1998	12264	79		0.7
1999	11072	-1192		9.7
2000	12249	1177		10.6
2001	11463	-786		6.4
2002	12265	802		7.0
2003	11801	-464		3.8
2004	11383	-418		3.5
2005	11771	388		3.4
2006	11643	-128		1.1
2007	11552	-91		0.8
2008	11612	60		0.5
2009	11473	-139		1.2
2010	11251	-222		1.9
2011	11163	-88		0.8
2012	11256	93		0.8
2013	11328	72		0.6
2014	11125	-203		1.8
2015	11037	-88		0.8
2016	10722	-315		2.9
2017	10466	-262		2.4

Table 3 - Annual wool sales and percentage change from one year to the next

Year	Merino wool sales (t)	Difference	Trend	Percentage Change
1980	70.2			
1981	75.6	5.4		7.7
1982	76.3	0.7		0.9
1983	71.6	-4.7		6.2
1984	69.6	-2.0		2.8
1985	65.5	-4.1		5.9
1986	60.8	-4.7		7.2
1987	61.3	0.5		0.8
1988	65.6	4.3		7.0
1989	68.9	3.3		5.0
1990	74.6	5.7		8.3
1991	58.4	-16.2		21.7
1992	53.7	4.7		8.1
1993	50.3	-3.4		6.3
1994	43.6	-6.7		13.3
1995	45.2	1.6		3.7
1996	43.0	-2.2		4.9
1997	39.8	-3.2		7.4
1998	39.4	-0.4		1.0
1999	35.3	-4.1		10.4
2000	33.5	-1.8		5.1
2001	33.2	-0.3		0.9
2002	31.5	-1.7		5.1
2003	32.8	1.3		4.1
2004	32.5	-0.3		0.9
2005	31.8	-0.7		2.2
2006	32.8	1.0		3.1
2007	32.5	-0.3		0.9
2008	31.5	-1.0		3.1
2009	32.9	1.4		4.4
2010	31.5	-1.4		4.3
2011	30.1	-1.4		4.4
2012	33.0	2.9		9.6
2013	33.6	0.6		1.8
2014	33.4	-0.2		0.6
2015	31.4	-2.0		6.0
2016	32.3	0.9		2.9
2017	18.0	-14.3		44.3

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Table 4 - Annual wool value and percentage change from one year to the next

Year	Wool value (R)	Difference	Trend	Percentage Change
1980	161099.3			
1981	222385.1	61285.8		38.0
1982	214381.6	-8003.5		3.6
1983	230485.9	16104.0		7.5
1984	320593.0	90107.1		39.1
1985	339631.3	19038.3		5.9
1986	325002.7	-14628.6		4.3
1987	575905.7	250903.0		77.2
1988	785480.4	209574.7		36.4
1989	699624.2	-85856.2		10.9
1990	521393.0	-178231.2		25.5
1991	441703.7	-79689.3		15.3
1992	340583.6	-101120.1		22.9
1993	343572.1	2988.5		0.9
1994	521725.2	178153.1		51.9
1995	452139.7	-69585.5		13.3
1996	545368.7	93229.0		20.6
1997	524802.4	-20566.3		3.8
1998	411997.2	-112805.2		21.5
1999	439955.3	27958.1		6.8
2000	519995.9	80040.6		18.2
2001	798693.4	278697.5		53.6
2002	1069361.2	270667.8		33.9
2003	769658.9	-299702.3		28.0
2004	646769.4	-122889.5		16.0
2005	614924.8	-31844.6		4.9
2006	951667.9	336743.1		54.8
2007	1212695.3	261027.4		27.4
2008	931227.7	-281467.6		23.2
2009	1178772.9	247545.2		26.6
2010	1373248.7	194475.8		16.5
2011	1788030.3	414781.6		30.2
2012	2048430.2	260399.9		14.6
2013	2266820.0	218389.8		10.7
2014	2293887.1	27967.1		1.2
2015	2736102.2	442215.1		19.3
2016	3034218.5	298116.3		10.9
2017	3408712.6	374494.0		12.3

Table 5 - Annual auction price and percentage change from one year to the next

Year	Auction price (R/kg)	Difference	Trend	Percentage Change
1980	2.29			
1981	2.94	0.7		28.4
1982	2.81	-0.1		4.4
1983	3.22	0.4		14.6
1984	4.6	1.4		42.9
1985	5.18	0.6		12.6
1986	3.35	-1.8		35.3
1987	9.39	6.0		180.3
1988	11.98	2.6		27.6
1989	10.15	-1.8		15.3
1990	6.98	-3.2		31.2
1991	7.56	0.6		8.3
1992	6.34	-1.2		16.1
1993	6.83	0.5		7.7
1994	11.97	5.1		75.3
1995	10.01	-2.0		16.4
1996	12.68	2.7		26.7
1997	13.19	0.5		4.0
1998	10.45	-2.7		20.8
1999	12.45	2.0		19.1
2000	15.52	3.1		24.7
2001	24.05	8.5		55.0
2002	34	10.0		41.4
2003	23.49	-10.5		30.9
2004	19.92	-3.57		15.2
2005	19.36	-0.6		2.8
2006	29	9.6		49.8
2007	37.31	8.3		28.7
2008	29.56	-7.8		20.8
2009	35.84	6.3		21.2
2010	43.58	7.7		21.6
2011	59.38	15.8		36.3
2012	61.88	2.5		4.2
2013	68.16	6.3		10.2
2014	68.63	0.4		0.7
2015	87.57	18.9		27.6
2016	93.8	6.2		7.1
2017	113.37	19.6		20.9

Regression analysis between parameters

The regression analysis between number of years and independent variables (annual Merino sheep numbers, wool sales and auction price) shown in Table 6. Four goodness of fit criteria (R^2, RMSE, AIC and BIC) were used to select the best regression model. The results indicated that all the predicted regression models were statistically significant at $P < 0.01$. The results of this study showed that quadratic model ($R^2 = 90$) was the best model to predict the relationship between number of years and annual Merino sheep numbers. Our results are in agreement with the findings of Tyasi (2021) who revealed that regression analysis suggested that quadratic model is the best fit model to explain the trend in both annual number of Angora goats and mohair production in South Africa between 1981 and 1987. Furthermore, exponential model showed a higher R^2 (0.86) and lower RMSE (0.02) for the estimation of association between number of years and wool sales. Moreover, our findings are agreement with results of Keskin et al. (2009) in Konya Merino sheep

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who revealed that quadratic and gompertz models showed the best fit to growth of Konya Merino lambs by having higher R^2 values, lower MSPE and non-auto correlation. The findings further recognized that power model with R^2 of 0.94, RMSE of 0.07, AIC of -99.41 and BIC of -96.19 was the best model to explain the relationship between number of years and auction price. Keskin et al. (2009) further revealed that these models can be used for predicting live weight at later ages from early partial live weight data.

Table 6 - Regression models to describe the relationship between parameters

Model	Equation	R^2	RMSE	AIC	BIC	P-value
Between number of years and annual Merino sheep numbers						
Linear	Y=492275-23943X	0.84	1421428.74	540.30	543.57	0.001
Logarithmic	Y=4E+06-5E+05ln(x)	0.84	1411295.33	540.03	546.43	0.001
Quadratic	$Y=3E+07-28110x+6.97x^2$	0.90	1421428.74	540.30	543.57	0.001
Exponential	$Y=6E+18e^{-0.02x}$	0.86	0.01	-193.53	-190.26	0.001
Power	$Y=3E+115x^{-33.74}$	0.86	0.01	-193.53	-190.26	0.001
Between number of years and wool sales production						
Linear	Y=2829.4-13929x	0.84	46.75	148.05	151.32	0.001
Logarithmic	Y=21216-2786ln(x)	0.84	46.42	147.78	151.05	0.001
Quadratic	$Y=158350-157.04x+0.04x^2$	0.84	46.75	148.05	151.32	0.001
Exponential	$Y=8E+27e^{-0.03x}$	0.86	0.02	147.78	-144.51	0.001
Power	$Y=1E+201x^{-60.45}$	0.86	46.75	-148.33	-144.51	0.001
Between numbers of years and auction price						
Linear	Y=4354.2+2.1921x	0.74	216.91	206.37	209.64	0.001
Logarithmic	Y=-33226+4375.4ln(x)	0.74	218.45	206.63	209.91	0.001
Quadratic	$Y=455063-457.58x+0.115x^2$	0.74	215.37	206.10	209.37	0.001
Exponential	$Y=2E-81e^{0.09x}$	0.94	0.07	-96.28	-96.13	0.001
Power	$Y=0x^{188.69}$	0.94	0.07	-99.41	-96.13	0.001

CONCLUSIONS

The current study focuses on characterization of the trends for annual Merino sheep numbers, wool sales and wool auction price in South Africa from 1980 to 2017. The study concluded that there was a trend in annual number of Merino sheep, wool sales and wool auction price from 1980 to 2017. All the regression techniques used to predict the relationships between numbers of year with annual number of Merino sheep, wool sales and wool auction price were statistically significant. The study concluded that quadratic, exponential and power models were the best fit models to predict the relationship of number of years with annual number of Merino sheep, wool sales and auction price respectively. The current study might help Merino sheep farmers for annual wool production planning.

DECLARATIONS

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Authors' contribution

Lubabalo Bila designed the manuscript, analysed data and wrote the final manuscript. Thobela Louis Tyasi supervised the manuscript and edited the final manuscript. Zenzile Peter Khetsha revised the manuscript. All the authors read, revised, and approved the final manuscript.

Conflict of interest

Authors declare no conflict of interest.

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CURRENT ATTITUDES AND SELF-RATED ABILITIES TOWARD PAIN ASSESSMENT OF HORSE OWNERS IN BOSNIA AND HERZEGOVINA

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ABSTRACT: Over the past decades, recognised importance of prompt and valid pain recognition and quantification in veterinary medicine significantly increased interest in investigating attitudes and self-rated abilities of veterinarians towards pain assessment. However, giving that the owners are the ones who decide when to call the veterinarian, it is also essential to investigate their attitudes and knowledge regarding pain recognition and management. This is the first research investigating horse owners' attitudes and self-rated abilities towards pain assessment and management in Bosnia and Herzegovina. The participants were invited via email or social media with attached link to online questionnaire created using the Google Forms platform. Surveyed population included adult sport or pleasure horse owners, and horse caretakers in Bosnia and Herzegovina. Study response rate was 33.33% (40 respondents). The questionnaire consisted of sections asking about respondents' demographic data, general pain assessment and management, and attitudes towards pain assessment done by veterinarians compared with theirs. Study respondents considered their abilities for pain recognition and quantification as sufficient and sufficient/moderate, even though most of them were unfamiliar with pain scales, and only negligible number use them. This study indicates the need and importance of owners' education concerning pain recognition and quantification in horses, and their more effective communication with veterinarians.

Keywords: Horse, Pain recognition, Pain quantification, Pain scales, Questionnaire.

INTRODUCTION

Accurate recognition of painful conditions in animals is crucial in treatment decisions (De Grauw and van Loon, 2016). In some animals, pain detection can be challenging as they are non-verbal (Fureix et al., 2010; De Grauw and van Loon, 2016). Reporting of the pain, same as the diseases, by owners is affected by their understanding and recognition of clinical signs. The owner's responsibility is to recognize the disease promptly and to find professional help providing a comprehensive animal history (Ireland et al., 2012). Some conclusions of previous studies point out that owners of geriatric horses recognize clinical signs but misinterpret them as normal or fail to link them to the disease (McGowan et al., 2010; Ireland et al., 2012). These omissions delay the veterinary treatment (Ireland et al., 2012). Earlier and more accurate recognition of the disease will lead to a better outcome for the horse (Scantlebury et al., 2014). In a study of Bowden et al. (2020), even though owners showed interest for health assessment of their horses and were self-confident doing it, those assessments could not always be considered as reliable because of their insufficient knowledge. The above emphasise importance of adequate owners' education and timely communication with veterinarians. There is one study investigating owners' attitudes toward pain recognition in bovine (Tschoner et al., 2021), and to the best of our knowledge one study regarding owners' pain recognition in horses in which owners and veterinarians pain assessments differed significantly (Sellon et al., 2022).

This study aims to identify and evaluate attitudes towards pain by horse owners and caretakers in Bosnia and Herzegovina, and to assess the need for educational programs establishment for equestrian staff to improve welfare and horses' quality of life.

MATERIALS AND METHODS

Study participants were invited to participate via email or social media (Facebook, websites dedicated to horse enthusiasts). The questionnaire was created as an online survey using the Google Forms platform, and its completion was voluntary and anonymous. The study population included adult sport or pleasure horse owners and horse caretakers in Bosnia and Herzegovina. Questions were based on previously published studies (Capner et al., 1999; Lascelles et al., 1999; Raekallio et al., 2003; Hugonnard et al., 2004; Williams et al., 2005; Lorena et al., 2013; Beswick et al., 2016). The four-sectioned questionnaire included questions to assess horse owners' attitudes towards pain and pain management in

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their horses. The questionnaire's first section consisted of questions about respondents' demographic data. The second section contained questions about general pain assessment, the ability of the respondents to detect and quantify pain, assessment methods, and factors influencing their assessment. The ability to detect and quantify pain was classified as excellent, sufficient, moderate, and insufficient, cannot assess, and never assess pain. Questions about the use of behavioural and physiological parameters for pain assessment, as well as knowledge of different pain assessment tables and their use were contained in the third section of the questionnaire. The fourth part consisted of questions related to pain management and attitudes of the respondents towards pain assessment done by veterinarians compared with theirs. At the end of the questionnaire, horse owners and caretakers had the opportunity to leave their comments, opinions, and recommendations related to this topic.

Ethical regulation

The study was approved by the Ethics committee of the Veterinary Faculty University of Sarajevo under approval number 01-02-18-5/20.

RESULTS

The total number of completed questionnaires in this study was 40 (33.33%) out of total of 120. Most study respondents, a total of 29 (72.00%), were under 30 years of age, while the remaining respondents, a total of 11 (28.00%), were younger than 50 years. Considering gender of the respondents, 22 (55.00%) were men and 18 (45.00%) were women. That pain was difficult to assess is considered by 25 (62.50%) of the respondents. Almost all respondents, 38 (95.00%) assess pain in horses. Respondent's self-rated abilities for pain detection and quantification are presented in Figure 1.

Almost all respondents who are assessing pain, 27 (71.05%), are not familiar with the scales for pain assessment in horses. Pain assessment scales were used by 3 (7.89%) study respondents. All study respondents agreed that knowing the animal's typical behaviour facilitates pain assessment. The intensity of manifested symptoms is considered to be primarily under the influence of individual sensitivity, 28 (70.00%) of the respondents, animal species 15 (37.50%), previous pain experience and environment 13 (32.50%), and animal breed 7 (17.50%). The behaviours based on which participants assess horse pain are shown in Table 1. That they could reliably estimate heart rate is considered by 26 (65.00%) of the study respondents, respiratory rate 32 (80.00%), digestive sounds 28 (70.00%), and rectal temperature 29 (72.50%).

Physiological parameters as indicators of pain were used by 24 (63.15%) of the study respondents. That owner can reliably assess and interpret behaviours caused by pain is considered by 26 (65.00%) of the study respondents, while 23 (57.50%) consider that owners can better notice the presence of pain in their animals than a veterinarian. The majority of respondents, 37 (92.50%), believe that analgesia is a significant benefit of pain management. Although 34 (85.00%) participants believe that the animal's recovery will be better if analgesia is present, 32 (80.00%) still consider that a certain degree of pain is beneficial because it reduces animal activity during a painful condition. If they notice pain in their animal, 22 (55.00%) study respondents will insist on the use of analgesics.

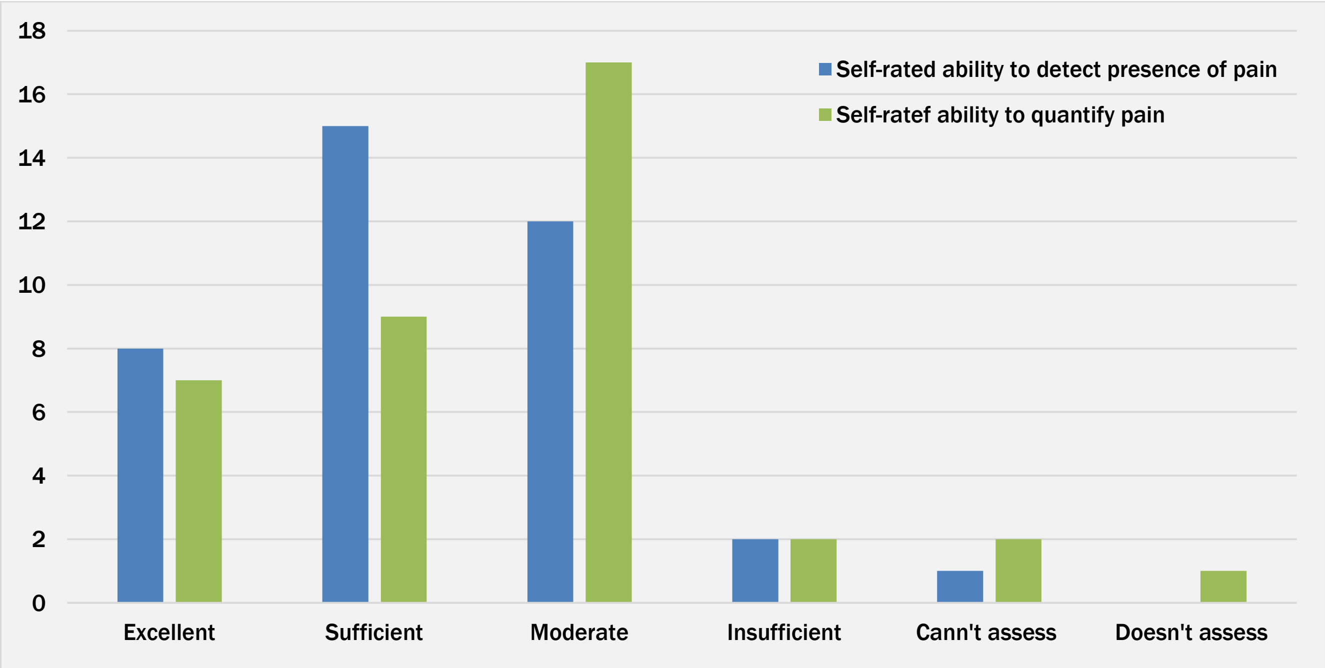

Figure 1 - Respondents' self-rated ability to detect presence of pain and its quantification in numerical analysis (numbers)

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Table 1 - Ranking of behavioural pain indicators in horses used by respondents

Behavioural pain indicators	Number citing (%)
Facial expresions	32 (84.21)
Demeanour	30 (84.21)
Response to palpation of painful area	28 (73.68)
Appetite	28 (73.68)
Sweating	22 (57.89)
Posture (weight distribution, comfort)	20 (52.63)
Rolling	19 (50.00)
Head movements	18 (47.36)
Kicking in the abdomen	18 (47.36)
Collapse	17 (44.73)
Interactive behaviour (reaction to observer(s))	17 (44.73)
Interactive behaviour (aggression towards handlers)	16 (42.10)
Pawing	16 (42.10)
Interactive behaviour (aggression towards foal and other horses)	13 (34.21)
Tail flicking (excluding flicking to chase off insects)	11 (28.94)
Tooth grinding	11 (28.94)
Tears	10 (26.31)
Interactive behaviour (reaction to sound stimulus)	10 (26.31)
Vocalization	9 (23.68)
Stretching	7 (18.42)
Lateral recumbence	4 (10.52)
Stupor	4 (10.52)

DISCUSSION

This is the first report from Bosnia and Herzegovina (B&H) regarding owners' attitudes toward pain in domestic animals. The main goal was to investigate the attitudes of horse owners toward pain recognition, its assessment, and management. There is no official horse owner's database in B&H. Therefore, respondents were identified and reached in various ways, which included veterinarians, horse shows and competitions, and horse breeders. According to the official data of the Agency for Statistics of B&H, in 2019 the number of horses was 14,000. The number of horses in 2019 decreased by 5% compared to the previous year, although there is an increase in the number of sports and recreational horses according to the authors' knowledge.

Considering previous similar survey studies (Price et al., 2002; Williams et al., 2005; Tomsič et al., 2021) lower response rates are not unusual. However, it doesn't mean that results of studies with low response rate are not representative (Stang and Jöckel, 2004; Hotchkiss et al., 2007). Most study respondents assess pain, and find it difficult to assess, but were self-confident about their abilities for pain recognition and quantification. Similar results were in a study examining the attitudes of veterinarians in B&H towards pain recognition and quantification, were most of the respondents considered their knowledge as sufficient in pain recognition and sufficient and moderate for pain quantification (unpublished authors data). Similar findings were reported from studies conducted in New Zealand (Williams et al., 2005), Ontario (Beswick et al., 2016), and in Colombia (Morales-Vallecilla et al., 2019). French (Hugonnard et al., 2004) and Swiss (Perret-Gentil et al., 2014) veterinarians considered their abilities as inadequate for pain quantification. Most study respondents are not familiar with pain assessment scales, as well as veterinarians in BiH (unpublished authors data).

The situation is similar with veterinarians in France (Hugonnard et al., 2004) and Queensland (Weber et al., 2012). Although it has been proven that pain assessment scales increase the ability of observers to recognize and quantify pain in animals (Morales-Vallecilla et al., 2019). Possible explanation for this could be the lack of available literature about pain recognition, assessment and pain scales on Bosnian language. Veterinarians in Colombia who participated in continuous training programs were more likely to use pain scales (Morales-Vallecilla et al., 2019), which indicates the need and benefit of such programs, attended by not only the veterinarians, but also animal owners because since they will decide when to seek professional help for their animal. If they notice pain in their animals, more than half of the respondents, will insist on the use of analgesics, emphasizing the importance of adequate and timely pain recognition and quantification done by owners. In a study of Tschoner et al. (2021), farmers and veterinarians estimated pain intensity similarly, but recognized pain differently and disagreed in opinions regarding analgesics use. Therefore, to ensure adequate pain management communication between both groups should be improved (Tschoner et al., 2021). All respondents believed that pain assessment is facilitated by knowing animal typical behavior, as well as veterinarians in B&H (unpublished authors data), and accordingly, most of them agreed that individual sensitivity has the greatest impact on its manifestation. The majority of owners felt that they could notice pain in their animals better than the veterinarian

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and that their pain assessment is reliable, contrary to the opinon of veterinarians in B&H (unpublished authors data). Similary as veterinarians in Finland (Raekallio et al., 2003), even though it is believed that animal owners and keepers can recognize behavioral changes that would otherwise go unnoticed, but don't necessarily interpret them as pain indicators. The study of Bornmann et al. (2021) indicate that some equestrians have too much self-confidence in interpreting affective states of horses, i.e. some participants incorrectly assessed behaviors that potentially indicate negative emotions, such as pain or distress, as indicators of happiness. This misperception can jeopardize the well-being of the horse as well as the rider's safety (Bornmann et al., 2021). Even with previous experience with the disease, most owners failed to recognize its signs (Scantlebury et al., 2014).

Facial expressions were the most commonly used pain indicator by respondents. In addition to facial expressions, respondents relied on demeanor, response to the touch of the painful area, and appetite for pain assessment. For comparison, veterinarians in B&H listed demeanour as the most useful behaviour when assessing pain (unpublished authors data). Only one-fifth of the respondents in this study considered vocalization as an indicator of pain, while it was considered as an important indicator of pain by BiH veterinarians (unpublished authors data), similar to the opinions of veterinarians in Ontario (Beswick et al. 2016), and in contrast to French (Hugonnard et al., 2004) veterinarians. In a study conducted by Tschoner and co-workers veterinarians used parameters such as bruxism, vocalization (moaning), and increased respiratory rate, which may be less obvious signs of pain for farmers, who relied more on parameters such as reduced food intake, weight loss, and fever (Tschoner et al., 2021). Most of the respondents in this study considered that they can assess heart rate and respiration, digestive sounds, and measure body temperature, which they also use to assess pain. Similar results were recorded in study by Bowden et al. (2020), but the results showed that their assessment of these parameters and knowledge about their normal values was not always correct. Almost all respondents in this study considered that animals have benefits of pain relief, as well as that the recovery is better if they received analgesics. Curiously, simultaneously the majority of respondents considered that a certain degree of pain is useful because it reduces animals' activity during painful conditions or after surgery. This observation is comparable to the opinions of veterinarians in B&H, the UK, New Zealand, and Finland (Capner et al., 1999; Raekallio et al., 2003; Williams et al., 2005). In a study conducted in Denmark, farmers considered most diseases to be more painful than veterinarians, but were less likely to use analgesics (Thomsen et al., 2012).

Most farmers in Bavaria were concerned about possible side effects of analgesics after their application to livestock, and this opinion can be the reason for farmers 'attitudes to administer fewer analgesics to livestock during and after procedures, compared to veterinarians (Tschoner et al., 2021). The use of other pain-relieving methods, apart from analgesics, is still not present enough in BiH. In this study, owners considered that their knowledge about pain recognition and quantification, on a scale from 1 to 5, is moderate (graded as 3 and 4).

CONCLUSION

This is the first report regarding attitudes of B&H horse owners toward. Horse owners have shown interest for pain assessment and its management, and its impact on horse's welfare. Study respondents used the most facial expressions, demeanour, response to the touch of the painful area, and appetite for pain assessment. Pain scales, similar to veterinarians in BiH, were used by only negligible small number of respondents. Previous reports, as well as this, emphasize the importance and need for additional education tailored to horse owners on the importance of timely and accurate pain recognition and its management, its impact on the animal welfare, as well as its potential effect on the safety of people working with those animals.

DECLARATIONS

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Authors' contribution
All authors contributed equally to this work.

Conflict of interests
The authors have not declared any conflict of interests.

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VALUE-ADDED ANALYSIS OF THE MEAT AGROINDUSTRY IN INDONESIA

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ABSTRACT: This study analyzes the added value of processing fresh beef into beef jerky, shredded beef, and se'i (smoked beef) products in Kefamenanu. The research was conducted in February and March 2021. The research method used was the survey method. Data was collected from cattle slaughterers (butchers) in abattoirs (n = 7), meat retailers (n = 13), and MSMEs in the meat processing industry (n = 15) and consumers (n = 90). The sample was selected through a purposive sampling method, with the criteria for selecting a sample of MSMEs in the processing industry as follows: 1) entrepreneurs have beef jerky, shredded beef, and se'i (smoked beef); 2) entrepreneurs who have sold their products in the past year and their three products are circulating in the market; 3) entrepreneurs produce these three products sustainably. The data was analyzed using descriptive statistics, and the added values of beef jerky, shredded, and se'i (smoked beef) products were calculated using the Hayami method. The results showed that each processing of one kilogram of fresh beef could produce 0.70 kg (shredded), 0.73 kg (jerky), and 0.68 kg (se'i). The added value obtained is USD 3,56 for shredded products, USD 4,03 for jerky products, and USD 2,91 for se'i products. The profit from shredded beef is USD 3,34, with beef jerky of USD 3,80 and se'i (smoked beef) of USD 2,64.

Keywords: Beef fresh, Beef product, Farm management, Meat processing, Se'i, Value added.

INTRODUCTION

The role of the livestock sub-sector is becoming increasingly essential and strategic with the increasing demand and per capita consumption of various livestock products. This significant increase in demand for livestock products is thought to be due to the increasing population, income level, changes in lifestyle, especially in consumer tastes, and the increase in the number of industrial-scale livestock processing businesses. One form of the downstream industry in the livestock sector is the meat processing industry (Kadju et al., 2020). Meat is an essential food ingredient in meeting nutritional needs and also has high protein content with complete and balanced essential amino acids. Another advantage of meat protein is that it is easier to digest than vegetable protein. Meat contains several types of minerals and vitamins. Therefore, to compensate for many meat processing industries, it is necessary to analyze the condition of the availability of meat both in quality and quantity (Putri and Lamusa, 2017).

Kefamenanu city is the seat of government and the capital of the district of North Central Timor (TTU), which is astronomically located between 9°0'06" and 9°39'41" South Latitude and between 124°05'36" and 124°51'14" East Longitude. TTU Regency itself is one of the districts directly adjacent to the Democratic Republic of Timor Leste (RDTL), with an area of 2,669.70 km^2, or only about 5.6% of the land area of East Nusa Tenggara province (NTT), and is one of the national beef cattle producing areas that annually sends Balinese cows for beef needs to Samarinda city, DKI Jakarta, and West Java. Cattle slaughter activities in slaughterhouses (RPH) tend to increase with a growth rate of 3.89%. 1,528 cows were cut in 2015 and rose to 1,960 cows in 2019 (BPS) (TTU, 2020).

The number of beef cuts continues to increase due to beef consumption. This is to increase the demand for beef in the TTU district every year. The increasing proportion of beef consumption is due to easy availability, better public consumption awareness, and the development of agroindustry in the form of culinary businesses (houses providing beef-based menus) and the needs of home industries that specifically produce processed beef products. The beef processing industries that have developed well are the Shredded, Jerky, and Se'i (smoked beef) manufacturing industries. Beef processing industry players based in Kefamenanu city are downstream beef cattle agroindustry that produces ready-to-eat products directly consumed by consumers and can be stored longer. Therefore, it is necessary to focus on supply chain management to provide fast and cheap products and prevent product vacancies from reaching consumers (Febrianto et al., 2020; Ardiansyah et al., 2020).

Added value is a change in value that occurs due to the treatment of inputs in a production process. Increased value-added agricultural commodities flow upstream to downstream in each supply chain, beginning with the farmer and

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ending with the final consumer (Coltrain et al., 2000). Added value varies depending on input and treatment by each member of the supply chain (Setiadi et al., 2018; Lestari et al., 2019). The current state of customers has become connected, informed, and active rather than isolated and passive as it was a few years ago (Agapitou et al., 2017). The core principle of business is customer orientation, and the success of the company depends on effectively managing the relationship with them (Nguyen et al., 2007).

Another purpose of making jerky, shredded, and se'i (smoked meat) beef is to meet the community's nutritional needs because jerky, shredded, and se'i beef as foodstuffs from livestock are rich in nutrients (Syarif et al., 2013). Agroindustry downstream activities also aim to increase the added value of fresh beef products. These three products (jerky, shredded, and se'i) are currently the superior products of meat processing MSMEs that are always awaited to be enjoyed by the people of Kefamenanu city. Until now, there has been no research on the added value of processed products, so it is necessary to analyze the amount of added value generated from processing beef into jerky, shredded, and se'i in Kefamenanu city.

MATERIALS AND METHODS

The research was conducted from February to March 2021 in Kefamenanu city, North Central Timor Regency, East Nusa Tenggara. The research method used is the survey method (Singarimbun and Effendi, 2006). Data was collected from livestock-cutting traders (pejagal) in abattoirs (n = 7), meat retailers (n = 13), MSMEs in the meat processing industry (n = 15) and consumers (n = 90). Samples are selected through the purposive sampling method (Campbell et al., 2020), with the criteria for selecting samples of MSMEs in the processing industry being as follows: 1) entrepreneurs have jerky, shredded, and se'i (smoked beef) products; 2) entrepreneurs who have sold their products in the past year and all three of their products are on the market; 3) entrepreneurs produce all three products on an ongoing basis. Snowball sampling follows the instructions of Drăgan and Isaic-Maniu (2012) to determine the participants involved in the production process, ranging from the provision of meat raw materials (cutting merchants) in RPH to product buyers (consumers).

Data collection is carried out by in-person interviews with respondents using questionnaires that have been prepared. Data collection in this study includes primary data and secondary data (Sugiyono, 2017). Primary data is data obtained through in-person interviews with respondents. The secondary data, namely data obtained from the TTU Regency Livestock Office, the TTU Regency Industrial and Trade Office, the Central Statistics Agency of TTU Regency, and several related data sources that support the research's substance, is analyzed using descriptive analysis methods. The Hayami method calculates the added value of jerky, shredded, and se'i (smoked beef). The value-added criteria follow the below equation.

VA = VPP - (VRM + VSM)

Where: VA= Value-added (IDR/kg); VRM = Value of raw materials (IDR/kg); VSM = Material support value (IDR/kg); VPP = Value of processed product (IDR/kg)

The calculation procedure for value-added analysis uses the Hayami method (Table 1).

Table 1 - Stages of calculation of value added Hayami method (modified)

Variable		Value
Output, Input and Price	1) Output (kg)	(a)
	2) Raw material input (kg)	(b)
	3) Labor input (DWH)	(c)
	4) Conversion factors	(d) = (a) / (b)
	5) Field labor coefficient (DWH/kg)	(e) = (c) / (b)
	6) Output price (USD/kg)	(f)
	7) Average labor wage (USD/DWH)	(g)
Profit and Revenue (IDR/kg Raw Material)	8) Input price (USD/kg)	(h)
	9) Transaction fees (USD/kg)	
	10) Output value (USD/kg)	(j) = (d) x (f)
	11) a. Added value (USD/kg)	(k) = (j)-(i)-(h)
	b. Value-added ratio (%)	(l) = (k)/(j)
	12) a. Labor income (USD/kg)	(m) = (e)x(g)
	b. Labor incentives (%)	(n) = (m)/(k)
	13) a. Profit (USD/kg)	(o) = (k)-(m)
	b. Profit rate (%)	(p) = (o)/(j)
Production Factor Services (IDR/kg of Raw Materials)	Margin (USD/kg)	(q) = (j)-(h)
	a. Direct labor income (%)	(r) = (m)/(q) x 100
	b. Other input donations (%)	(s) = (i)/(q) x 100
	c. Company profits (%)	(t) = (o)/(q) x 100

Source: Hayami et al., (1987); DHW= Daily human works; USD= American dollar (value of 1 USD exchange rate IDR 14,500)

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RESULTS AND DISCUSSION

Beef shredded products' added value

Added value is a change in value that occurs due to changes in input in a production process (Setiadi et al., 2018). Technical factors that affect added value are production capacity, the number of raw materials used, and labor. At the same time, the market factors that affect output are the price of output, work wages, the price of raw materials, and the value of other inputs. The concept of "added value" is highly dependent on existing demand and often changes based on the values in a product that consumers want.

The value-added analysis of beef shredded products is per 1-time production; the standard price used for raw materials, labor, and other input donations is the price at the processing or producer level. The average calculation of the added value obtained by MSMEs from shredded products is presented in Table 2.

Table 2 - Average Value Added of Beef Shredded, Jerky and Se'i products in Kefamenanu city in 2021

Variable		Value		
		Shredded	Jerky	Se'i
Output, Input and Price	1) Output (kg)	35	55	25
	2) Raw material input (kg)	50	75	30
	3) Labor input (DWH)	2,43	3.20	1,86
	4) Conversion factors	0,70	0.73	0,68
	5) Field labor coefficient (DWH/kg)	0,049	0.043	0,074
	6) Output price (USD/kg)	13,10	13,10	12,41
	7) Average labor wage (USD/DWH)	4,66	5,52	3,69
Profit and Revenue (IDR/kg Raw Material)	8) Input price (USD/kg)	5,52	5,52	5,52
	9) Transaction fees (USD/kg)	0,09	0,06	0,01
	10) Output value (USD/kg)	9,17	9,61	8,44
	11) a. Added value (USD/kg)	3,56	4,03	2,91
	b. Value-added ratio (%)	39%	42 %	34 %
	12) a. Labor income (USD/kg)	0,23	0,24	0,27
	b. Labor incentives (%)	6,4%	5,8 %	9,4%
	13) a. Profit (USD/kg)	3,34	3,80	2,64
	b. Profit rate (%)	36,6%	39,5 %	31,2%
Production Factor Services (IDR/kg of Raw Materials)	Margin (USD/kg)	3,66	4,09	2,92
	a. Direct labor income (%)	6,2%	5,8%	9,4%
	b. Other input donations (%)	1,8%	1,4%	0,4%
	c. Company profits (%)	92,0%	92,8%	90,2%

Source: Primary data processed (2021)

Table 2 shows that the conversion value for the shredded product is 0.70. The product conversion factor understands that every 1 kg of fresh beef input will produce a shredded output of 0.70 kg. The decrease in meat weight is due to shrinkage during the production process (Soeparno, 2009). The conversion factor value affects the output value (IDR/kg) of the product produced. The average main raw material (fresh beef) needed in one production process is 50 kg and will produce 35 kg of shredded products. The value-added activity requires input donations from other materials (supporting materials), with a total cost of USD 0,09 for each kilogram of input (main raw material) used. These supporting ingredients include peanuts, granulated sugar, salt, cumin, onions, garlic, coriander, and coconut.

The output value obtained using beef shredded products is USD 9,17/kg. The added value obtained is USD 3,56 with a ratio of 38.83% of the total output value. The income received by labor per kilogram of output amounted to USD 0,23 or 6,36% of the total added value. The profit value is obtained from added value minus direct labor income, so that the profit received by MSMEs for each kilogram of output is USD 3,34, or 36.36% of the total output value.

The margin value is the difference between the output value and the input price. For each cycle, the production cycle of shredded MSMEs gets a margin of USD 3,66/kg shredded. The margin value of 91.25% is profit. In comparison, the remaining (8.75%) is divided into 6.20% of the margin is direct labor income, and 2.55% is a contribution of other inputs (supporting material costs) incurred by MSMEs in producing shredded. This indicates that the greater the output produced, the greater the added value obtained, and the more efficient the producer in trying, the greater the workforce's competitiveness (Sumardi et al., 2017).

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Added value of jerky products

Beef jerky is a traditional beef processed product native to Indonesia that is important to develop because it can support nutrition improvement programs and as a source of protein (Satmalawati et al., 2017). Beef jerky is made from the main raw material of fresh beef (Lau et al., 2021). The process of making jerky at the commercial level is generally given the addition of nitrate or nitrite salts with the main purpose of producing a red color and serving as a preservative. The results showed that MSME actors avoid nitrates and nitrites because they can produce nitrosamine compounds that are mutagenic and carcinogenic, which endanger health, so it needs to be considered (Andrade et al., 2005; Santarelli et al., 2008). The application of liquid smoke in the manufacture of beef jerky can significantly affect water activity, microbes, color, and preferences (Rahayu et al., 2012).

Calculation of value-added analysis of jerky products is per 1-time production using standard prices of raw materials, labor, and other input donations at the processing or producer level. The average calculation of the added value obtained by MSMEs from jerky products is presented in Table 2.

Table 2 shows that the conversion value in beef jerky products is 0.73, meaning that every 1 kg of fresh beef input will produce jerky of 0.73 kg. The need to produce jerky consists of the primary raw material in the form of fresh beef amounting to 75 kg with a value of USD 413,79 and donations from other inputs (supporting material costs) in the form of spices, which are generally garlic, onions, coriander, pepper, acid, and sugar (Suryati et al., 2012) with a total cost of USD 0,06 per kilogram of input (primary raw material) used. The output value obtained by the jerky production business is USD 9,61/kg. The added value obtained is USD4,03 with a ratio of 42% of the total output value. The profit received by labor for each kilogram of output amounted to USD 0,24 or 5,8% of the total added value. The value of profit is obtained from added value minus direct labor income. The profit received by MSMEs for each kilogram of output is USD 3,80 or 39,5% of the total output value. The margin value is the difference between the output value and the input price. The margin value of MSME jerky production of USD 4,09/kg of jerky is divided into 92,8% margin is UKKM profit, 5,8% margin is direct labor income, and 1,4% is a contribution of other inputs (supporting material costs) issued by MSMEs.

Se'i (smoked beef) product added value

Making Se'i (smoked beef), there are several stages, namely the production of thick meat connective tissue, cutting to form elongated pieces similar to mine rope (called lalolak), curing, and smoking, and finally, ready to be consumed or packaged (Supit et al., 2013). The average calculations of added value obtained by MSMEs from se'i (smoked beef) products are listed in Table 2.

Based on Table 2, it can be seen that the conversion value in se'i (smoked beef) products is smaller than other products (shredded and jerky), which is only 0,68, which means that every 1 kg of fresh beef input will produce se'i of 0,68 kg. The price of fresh meat raw materials per kg is USD 5,52 with a beef requirement of 30 kg. Other input needs (salt, onion, flavoring, pepper, saltpeter, kesambi wood, and packaging/label) with a total cost of USD 0,01 for each kilogram of input. The output value obtained from se'i products is USD 8,44. The added value obtained is USD 2,91/kg se'i, with a ratio of 34% of the total output value. The workforce's profit per kilogram of output amounted to USD 0,27 or 9,4% of the total added value. The profit received from the se'i production business per kilogram of output is USD 2,64 or 31.2% of the total output value. MSMEs get a margin of USD 2,92/kg divided into 90.2% is the benefit of MSMEs, 9.4% is a direct labor benefit, and 0.4% is a contribution of other inputs (supporting material costs). If examined further, the distribution of added value for shredded, jerky, and se'i products in Tables 2, 3, and 4 indicates that the existence of MSMEs contributes to the increase in regional economic growth. This is indicated by the large labor ratio of only 6.3% (shredded), 5.8% (jerky), and 9.4% (se'i). This figure illustrates that the balance between labor income and capital income is smaller than the business owners' income share, which reaches 90.2%-92.8%.

Hasanah et al., (2015) explain that if the agro-industry has a high level of profit obtained (in percent), then the agro-industry is suitable for increasing regional economic growth; otherwise, if the agro-industry has a high share of labor, then the type of agro-industry is ideal for equalization of employment opportunities. If you want to increase opinion and market share, it is necessary to coordinate the flow of integrated information; there will be agreements and cooperation on product availability, quality, and price. Related to the current condition of the COVID-19 pandemic, the information exchange process also occurs online through social media (Sikone, at al., 2022). Typical social media platforms commonly used by business actors to market livestock products and exchange information are Facebook applications, WhatsApp, Instagram, online networking websites, and other start-up marketing models.

CONCLUSION

Processed meat products, involving shredded beef, beef jerky, and se'i provides added value for MSMEs. Each processing of one kilogram of fresh beef can produce 0.70 kg (shredded), 0.73 kg (jerky), and 0.68 kg (se'i). The difference in treatment in the manufacturing process of each product has an impact on the amount of depreciation value of the resulting product, and provided added value of USD 3,56 of shredded products; USD 4,03 of jerky products; and USD 2,91 of se'i products. The profit obtained by agroindustry actors from producing shredded beef was USD 3,34 for jerky products of USD 3,80 and se'i products of USD 2,64. To empower and strengthen MSMEs, there needs to be continuous coaching and assistance from relevant agencies to be independent, resilient, and superior in providing services for consumer satisfaction and creating more excellent market opportunities.

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Authors' contribution

HY Sikone conceptualizes research, collected and analyzed data, and written scripts. B Hartono, Suyadi, and HD Utami and BA Nugroho designed the research and evaluated the manuscript before submission.

Conflict of interests

The author has no possible conflicts of interest in this paper's research, authorship, or publishing.

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EFFECT OF STRAIN AND AGE OF LAYER CHICKENS ON PROXIMATE CONTENTS OF EGG YOLK AND ALBUMEN

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ABSTRACT: This research assessed the effects of strain and age on the proximate compositions of egg yolk and albumen of the domestic chicken. A total of 504 eggs were used in a 3 X 3 factorial experiment involving the Lohmann white, Lohmann brown and White Leghorn which were 31, 40 and 53 weeks old using a completely randomised design (CRD). Data obtained were subjected to the two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure. Differences in means were separated using the Tukey pairwise comparisons method at 5% level of significance. The results show that, eggs from the Lohmann layers have significantly more protein but lower fat content in the yolk than the White leghorn; while albumen protein was slightly higher in the White leghorn with lower albumen fat in the white strains than the Lohmann brown. Protein content of egg yolk significantly increased as the birds advanced in age but albumen protein was not affected largely by layers' age. Yolk fat significantly decreased as the birds grew but albumen fat was not substantially affected by age of the hens. There was significant effect of strain by age interaction on yolk and albumen protein contents but not on their fat content across the chicken groups. There are variations in the proximate contents of egg yolk and albumen of layer chickens by virtue of their genetic constitution and ages; so, eggs must be produced from the best strains at the appropriate hen-ages to make their nutrient contents meet the needs of specific consumers and products.

Keywords: Genetic constitution, Lohmann white, Lohmann brown, Variations, White Leghorn.

INTRODUCTION

Human population is expected to shoot up by 33% from 7.2 to 9.6 billion (UN, 2013) or 9 billion (International Egg Foundation-IEF, 2014) by 2050. Also, the world's standard of living has been projected to rise with about 70% increase in the demand for agricultural products around the same period (FAO, 2009). Animal products contribute nearly 17% and 33% of global kilocalorie and protein consumption respectively (Rosegrant et al., 2009). The chicken egg has formed significant part of human foods globally for many ages (Forson et al., 2011); providing high but cheap nutrition to the poor ones (Pascoal et al., 2008; Menezes et al., 2009). Eggs are the target in the IEF's self-sufficiency, independent and sustainable supply of food promotion; to provide low-cost vitamins, minerals and quality proteins for the present-day and the next generations (IEF, 2014). Earlier on, eggs were reported to be complete foods and the best choice for producing therapeutic diets for adult humans due to the low caloric content, good protein quality and high digestibility (Bufano, 2000; Song and Kerver, 2000). Despite these nutritional benefits, egg consumption in Ghana is about ten times lower than the world per capita average consumption (Ayim-Akonor and Akonor, 2014). This might be due to the misconceptions and panic tagged to eggs possibly due to misinformation on the nutritional and health benefits of eggs (Sass et al., 2018; Zhang et al., 2020). For instance, increased serum cholesterol and incidents of cardio-vascular diseases (CVDs) have been ascribed to eating eggs (Kritchevsky and Kritchevsky, 2000) and so, people fear to eat or increase its consumption.

Biochemical (nutritional) properties of egg such as protein, amino acids, lipid and minerals; fatty acids, vitamins, carotenoids, antioxidants, cholesterol (Rizzi and Marangon, 2012), carbohydrates (Huopalahti et al., 2007) and crude fibre (solids) are subjected to change due to differences in genetic constitution (Rizzi and Marangon, 2012; Youssef et al., 2014), and also age of layers (Kucukyilmaz et al., 2012). In most countries such as Ghana, greater part of table eggs is obtained from the domestic chicken with farmers developing interest for commercial or improved layers than the traditional lines (Aning, 2006). The Lohmann strains are becoming popular on Ghanaian farms though the biochemical or nutritional compositions of their eggs are not commonly known – which is substantiated by the idea that, paucity of information exists on the nutritional qualities of eggs from different breeds of the domestic chicken (Bashir et al., 2015). There is therefore the need to investigate the effects of genotype (strain) and age of layer chickens on the nutritional constitution of their eggs. Consequently, the objective of this study was to evaluate the effects of strain (genotype) and age on the proximate composition of egg yolk and albumen of selected layer chickens popularly found on Ghanaian

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farms. Such information could help farmers to select better strains for egg production and to collect eggs at appropriate bird ages for specific nutrient quality. The findings will also be useful for dieticians to prescribe quality eggs for people with peculiar nutritional challenges as well as enable industry to select eggs for specific products.

MATERIALS AND METHODS

Study areas and research design

Eggs used were obtained from the same farm in Abokobi located on latitude 5° 44´ N and longitude 0° 12´ W in the Greater Accra Region of Ghana. The experiment was conducted at the Nutrition Laboratory, Technology Village, University of Cape Coast (UCC) located on latitude 5.1036° N and longitude 1.2825° W. The Central region where UCC is found, is characterised averagely by minimum and maximum temperatures of 21 to 25°C and 26 to 32°C accordingly and 1300mm precipitation per year. A 3 X 3 factorial experimental design involving the Lohmann White, Lohmann Brown and White Leghorn that were 31, 40 and 53 weeks into lay in a completely randomised design (CRD) was used. The three-layer strains were kept in a deep litter house with same management practices carried out on them. 504 freshly laid eggs (168 from each strain and 56 eggs from each age group per strain) were randomly chosen and examined. Eggs that did not have their yolk well separated from the albumen were discarded. The birds were fed a layer mash with 18% crude protein and 3200kcal/kg ME. All essential vaccinations and medications were followed.

Data collection instruments, procedures and analysis

Eggs from the three chicken groups were randomly collected on the same day between 8:00 – 8:30am and once per each experimental week (hen-age), cleaned with a dry cloth, packed onto egg crates in a carton and sent to the laboratory by road for averagely four hours in transit. On arrival, the eggs were identified with a permanent marker and broken for experimentation within 24 hours. The eggs were broken with a scalpel onto a petri dish and the yolk completely separated from the albumen using a plastic yolk separator. All yolks as well as albumen per experimental unit were poured into one clean and sterilised beaker, labelled and centrifuged to homogenise. The samples were dried at 60°C in a Genlap Oven (Compact Test Equipment), grinded and refrigerated in zip lock bags at 8°C. Proximate analysis was done by the methods described by Horwitz and Latimer (2005). All equipment were sterilised before each experiment; but they were also regularly and thoroughly cleaned with distilled water and tissue paper after each experimental unit. All experiments were conducted at room temperature (19-22°C). Data collected were subjected to two-way ANOVA using the GLM procedure embedded in Minitab (version 18). Differences in means were separated using the Tukey pairwise comparisons method at 5% level of significance. The model used was:

$$Y_{ij} = \mu + S_i + A_j + (SA)_{ij} + \varepsilon_{ij}$$

Where: Y_{ij} = the dependent variable, μ = the general mean, S_i = ith observation of strain, A_j = jth observation of age, $(SA)_{ij}$ = interaction between strain and age and ε_{ij} = the random error associated with the dependent variable.

RESULTS

Table 1 displays the effect of strain of layer chickens on proximate composition of egg. The outcome shows significant genotype effect on protein and fat contents of yolks. Yolk from the Lohmann strains had more protein but lower fat content compared to the White leghorn. Though protein and fat contents of the albumen were not greatly influenced by strain, albumen protein was highest in the White leghorn (73.2%) followed by the Lohmann white (72.6%) and least in the Lohmann brown (71.7%); with lower fat in the albumen of eggs from the white strains (0.2%) than those from the Lohmann brown (1.8%). There was no significant strain effect on yolk ash but albumen ash was significantly influenced by strain with the white strains performing better than the Lohmann brown in both cases. Yolk fibre was significantly more in the white layers (0.8%) than the brown layers (0.7%). The Lohmann strains had significantly more albumen fibre (0.5%) than the White leghorn (0.4%). The carbohydrate content of both egg yolk and albumen were not greatly affected by the birds' strain. While the highest carbohydrate content of the yolk was recorded in the White leghorn, its composition in the albumen was insignificantly better in eggs from the Lohmann strains than the White leghorn.

The effect of age of layer chickens on proximate composition of eggs is presented in Table 2. The results show significant increases in protein content of egg yolk as the birds advanced in age from week 31 (30.6%) to week 40 (31.6%) though it increased insignificantly to 32.1% at week 53. Although albumen protein was not affected largely by the layers' age, there were slight differences with the highest at week 40 (72.7%) followed by week 53 (72.5%) and least during week 31 (72.0%). There was significant difference in yolk fat between the youngest age group at week 31 (52.0%) and the older groups at weeks 40 (51.0%) and 53 (50.8%) indicating a decrease in yolk fat as the chickens aged. However, albumen fat was not substantially affected by age though it was lower during the younger ages (0.2%) than the oldest age at week 53 (1.9%). The ash components of the yolk and albumen were significantly influenced by layers' age. The amount of ash in the yolk decreased from 3.4% (week 31) to 3.2% (weeks 40) then increased to 3.3% at week 53. Albumen ash was higher during the youngest and oldest ages (3.3%) compared to the mid-age group (3.2%). Fibre was not significantly influenced by age in both egg parts; but while albumen carbohydrate content was significantly affected by age, the differences were not substantial in the yolk.

Table 3 presents the effect of strain by age interaction of layers on proximate composition of egg. The results show significant effect of strain by age interaction on yolk protein but not albumen protein. The Lohmann white recorded the highest yolk protein content at week 40 followed by week 53 and the least at week 31. Meanwhile, albumen protein

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increased with increasing age in the Lohmann Brown strain. Yolk protein increased greatly as the Lohmann brown hens advanced in age while albumen protein of their eggs increased insignificantly as they grew. Protein content of yolk from the White leghorn was highest in the youngest group followed by the oldest group and least in the breed during the 40th week. The greatest albumen protein content of 73.6% was noted at week 40 followed by 73.0% at weeks 31 and 53 in the White leghorn chicken. Similarly, there was substantial effect of strain by age interaction on yolk fat but not albumen fat. The fat content of albumen (egg white) was approximately 0.2% among the chicken strains at all ages. On the other hand, yolk fat declined as the Lohmann White and Brown strains grew from the 31st week (52.2 and 52.3%) to the 40th week (50.8 and 50.9%) to the 53rd week (49.7 and 50.7%) respectively while the trait was highest in the egg component at week 53 (52.1%), followed by week 31 (51.6%) and lowest at week 40 (51.2%) in the White leghorn. The findings show significant interaction effect of chicken strain and age on ash content of both yolk and albumen. Yolk ash was higher at weeks 31 and 53 (3.4%) than week 40 (3.2%) but decreased in the albumen from 3.4% to 3.1% then increased to 3.3% at the ages respectively for the Lohmann white. Yolk ash in the Lohmann brown and White leghorn decreased with increasing age even though albumen ash showed an increasing trend. Both yolk and albumen had decreased ash content, as the White leghorn hens grew older. The strain by age interaction did not largely influence the fibre components of egg yolk and albumen with the compositions ranging from 0.76 to 0.8% and 0.5 to 0.6% according to the egg parts. Lastly, the carbohydrate contents of both egg parts were noticeably affected by the interaction.

Table 1 - Effect of strain of layers on proximate composition of egg

Nutrient (%)	Strain of layers	Lohmann White	Lohmann Brown	White Leghorn	SEM	P-value
Yolk	Protein	32.1^{a}	31.5^{b}	30.8^{c}	0.121	0.001
	Fat/oil	50.9^{b}	51.3^{ab}	51.6^{a}	0.181	0.014
	Ash	3.3^{a}	3.2^{a}	3.3^{a}	0.027	0.114
	Fibre	0.8^{a}	0.7^{b}	0.8^{a}	0.0051	0.020
	Carbohydrates	12.9^{a}	13.2^{a}	13.5^{a}	0.196	0.078
Albumen (egg white)	Protein	72.6^{a}	71.4^{a}	73.2^{a}	0.590	0.088
	Fat/oil	0.2^{a}	1.8^{a}	0.2^{a}	0.658	0.135
	Ash	3.3^{a}	3.2^{b}	3.3^{a}	0.030	0.003
	Fibre	0.5^{a}	0.5^{a}	0.4^{b}	0.005	0.001
	Carbohydrates	23.4^{a}	23.1^{a}	22.8^{a}	0.221	0.148

Means in rows with different superscripts are significantly different; SEM: Standard Error of Means; *p* < 0.05.

Table 2 - Effect of age of layers on proximate composition of egg

Nutrient (%)	Age (weeks)	31	40	53	SEM	P-value
Yolk	Protein	30.6^{b}	31.6^{a}	32.1^{a}	0.121	0.001
	Fat/oil	52.0^{a}	51.0^{b}	50.8^{b}	0.181	0.001
	Ash	3.4^{a}	3.2^{b}	3.3^{b}	0.027	0.001
	Fibre	0.8^{a}	0.8^{a}	0.8^{a}	0.005	0.587
	Carbohydrates	13.2^{a}	13.4^{a}	13.0^{a}	0.196	0.470
Albumen (egg white)	Protein	72.0^{a}	72.7^{a}	72.5^{a}	0.590	0.709
	Fat/oil	0.2^{a}	0.2^{a}	1.9^{a}	0.658	0.705
	Ash	3.3^{a}	3.2^{b}	3.3^{a}	0.023	0.012
	Fibre	0.5^{a}	0.5^{a}	0.5^{a}	0.005	0.400
	Carbohydrates	24.0^{a}	23.4^{a}	21.8^{b}	0.221	0.001

Means in rows with different superscripts are significantly different; SEM: Standard Error of Means; *p* < 0.05.

Table 2 - Effect of strain by age interaction of layers on proximate composition of egg

Nutrient (%)		Protein	Fats/oil	Ash	Fibre	CHOs	Protein	Fats/oil	Ash	Fibre	CHOs
Strain of Layers	Age of Layers	Egg Yolk					Albumen (Egg White)				
Lohmann White	Week 31	30.4^{c}	52.2^{a}	3.4^{a}	0.78^{a}	13.2^{abc}	70.8^{a}	0.2^{a}	3.4^{a}	0.6^{a}	25.0^{a}
	Week 40	33.1^{a}	50.8^{bcd}	3.2^{c}	0.78^{a}	12.1^{c}	72.4^{a}	0.2^{a}	3.1^{c}	0.5^{a}	23.7^{abc}
	Week 53	32.8^{a}	49.7^{d}	3.4^{a}	0.78^{a}	13.3^{abc}	74.4^{a}	0.2^{a}	3.3^{ab}	0.5^{a}	21.5^{de}
Lohmann Brown	Week 31	30.3^{c}	52.3^{a}	3.4^{a}	0.77^{a}	13.2^{abc}	72.1^{a}	0.2^{a}	3.1^{c}	0.5^{a}	24.0^{abc}
	Week 40	31.5^{b}	50.9^{abcd}	3.2^{c}	0.76^{a}	13.6^{ab}	71.9^{a}	0.2^{a}	3.2^{bc}	0.5^{a}	24.2^{ab}
	Week 53	32.6^{a}	50.7^{cd}	3.2^{c}	0.77^{a}	12.8^{bc}	70.1^{a}	0.2^{a}	3.2^{bc}	0.6^{a}	20.9^{e}
White Leghorn	Week 31	31.0^{bc}	51.6^{abc}	3.4^{a}	0.8^{a}	13.2^{abc}	73.0^{a}	0.2^{a}	3.4^{a}	0.5^{a}	22.9^{bcd}
	Week 40	30.3^{c}	51.2^{abc}	3.3^{b}	0.8^{a}	14.3^{a}	73.6^{a}	0.2^{a}	3.3^{ab}	0.5^{a}	22.4^{cde}
	Week 53	30.9^{bc}	52.1^{ab}	3.2^{c}	0.8^{a}	13.0^{abc}	73.0^{a}	0.2^{a}	3.3^{ab}	0.5^{a}	23.0^{bcd}
SEM		0.209	0.313	0.046	0.009	0.339	1.020	1.140	0.052	0.008	0.034
P-value		0.001	0.001	0.004	0.967	0.002	0.097	0.078	0.003	0.683	0.001

Means within columns with different superscripts are significantly different; CHOs: carbohydrates; SEM: Standard Error of Means; *p* < 0.05.

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DISCUSSION

Preference for egg yolk and albumen may change from one consumer or product to another depending on chemical composition of these egg parts as well as the nutritional needs and health status of consumers. The findings of the study show that, the 30.8 – 32.1% crude protein found in the yolk among the strains is close to the 30% reported by Li-Chan et al. (2013) who worked on the chemistry of chicken eggs on the same dry matter basis. Again, the values obtained in the current research are within the results of Ebegbulem and Asukwo (2018) who reported protein values of 21.87-35.18% for various chicken eggs somewhere in Nigeria but lower than the 44% published by Jianping (2014). The trend noticed in the crude protein content of the yolk for the three strains in the present analysis aligns with that documented for the egg yolk of four different chicken strains (Delta, Hy-Line W-36, Hy-Line W-77 and White leghorn) by Ahn et al. (1997) and further corroborates the observations made by Bashir et al. (2015) for two different breeds of domestic chicken. The protein from the egg yolk and albumen of the three chicken strains (genotypes) could therefore be suitable for making pharmaceutical, cosmetic and biotechnological products (Laca et al., 2010). Nevertheless, albumen from the White leghorn is best recommended for consumers due to its high protein content.

The fat content of 50.9 to 51.6% found in the yolk among the chicken genotypes is far lower than the 65% yolk lipid content reported by Jianping (2014), which implies that, yolks from the layers (especially the Lohmann strains) could be good for people with cardiovascular challenges presumed to be caused by high fat content or high serum cholesterol (Kritchevsky and Kritchevsky, 2000; McGee, 2004; Spence et al., 2012; Ariza et al., 2021). The lower level of fat in the yolk observed in the Lohman strains than the White leghorn might be attributed to the fact that the former used more energy to form and lay eggs than the later (Roberts, 2004; Rizzi and Chiericato, 2010). The low-fat content of egg white from the chicken strains renders them good for people who suffer from elevated cholesterol level or makes them good for making weight-reducing diets (Bashir et al., 2015). Lipid contents found in the yolk (30.41%) and albumen (1.09%) of the domestic chicken (Bashir et al., 2015) are lower than respectively found for the egg components in the current work. The 1.8% fat content noted in the albumen of the brown layer falls within the 1-2% lipid content of food reported to be adequate for humans (Attia et al., 2014) while yolk fat among the three chicken strains is higher than the recommended range. The high protein to low fat content of the albumen concords to the 50% (protein): 0.03% (fat) contents recorded for the same egg part (Jianping, 2014) and makes eggs laid by each chicken strain good for people who need high protein but low-fat foods such as those suffering from elevated cholesterol level.

The similarity of ash content in the yolk among the three chicken genotypes though greater than the 0.5 – 0.6% reported (Sugino et al., 1997) and the higher albumen ash content of eggs in the White leghorn over those from the Lohmann brown disagree with Bashir et al. (2015) who recorded significantly higher ash content in the yolk of a hybrid chicken (3.42%) than the domestic chicken (1.50%). The higher fibre content found in the yolk than the albumen across the strains may be because the yolks were large – as bigger yolks are said to contain more total solids as submitted by Ahn et al. (1997) who also reported differences in yolk and albumen ash contents in some layer chicken genotypes. Albumen from the layers could be a good source of energy as relatively higher carbohydrate was detected compared to their yolk and hence, may be good for people during emergencies as eggs digest easily (Bufano, 2000). This however, does not concord to the lower carbohydrate (energy) component of albumen recently noted (Jianping, 2014; Abdul-Rehman et al., 2016). The carbohydrate contents of the egg parts among all the three chicken strains are also more than the 2.88 – 5.28% recorded for the trait in different laying species (Fakai et al., 2015) as well as the 9.28 and 5.81% noticed in indigenous and commercial chickens respectively by Chepkemoi et al. (2017). Results from the current data indicate that generally, eggs (yolk and albumen) from the Lohmann white chicken is the best regarding proximate compositions.

The increasing nature of protein content of egg yolk and albumen as the birds aged suggests that, eggs from older layers should be considered for people and products that require high amount of protein but eggs from the chicken at week 40 are most recommended. The inconsistency remarked in albumen protein with age harmonises with Senčić and Samac (2017) though their report was for the whole egg. The findings also show that, for high protein eggs, the layer chicken could be kept up to week 53 even though albumen protein could decrease at this age. In regards, care must be taken when keeping layer chickens beyond week 53 as Ahn et al. (1997) have found lower protein contents of 16.96 and 16.75% at weeks 55 and 78 respectively which was confirmed by a decrease in protein content of eggs from chicken with increasing age (Diaz et al., 2010). The reduction in yolk and albumen fat as the hens grew is in support of Senčić and Samac (2017) and suggests that, eggs from younger birds are more suitable for people that suffer from fat-related diseases. Meanwhile, the high fat content seen in egg yolk than the albumen at all ages of the birds is in line with Senčić and Samac (2017). Variation in yolk fat with hen-age agrees with Roberts (2004) and Sahan et al. (2014) though they reported a lower yolk lipid content of 30%. But in opposition Ahn et al. (1997) found yolk lipid content to increase from 30.70% (week 28) to 30.95% (week 55) even though their results also showed a declining trend in older layers at weeks 78 (30.66%) and 97 (30.61%). The current results are entirely inconsistent to the notion that, fat content of egg increases as hens get older due to increased yolk size and fat deposition in older hens (Sahan et al., 2014) but agree with the fact that lipid in egg white is negligible in older chickens (Thammarat et al., 2009). Per the findings of this study, layers can be kept in lay up to week 53 if lower egg fat is the target but measures must be put in place to avoid any increases. Information on the effect of layers' age on yolk and albumen ash contents are scanty; but Pambuwa and Tanganyika (2017) have reported insignificant increase in ash content of eggs produced by the Malawian normal feathered hens

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during weeks 20 (1.288%), 24 (1.296%) and 28 (1.394%) though their findings disagree with Diaz et al. (2010) who discovered a decrease in ash content of chicken (meat) with increasing age. Nonetheless, the later results is supported by the decreasing effect of old age on yolk and albumen contents of eggs collected between weeks 31 and 40 in this experiment. Notwithstanding these, there were inconsistencies in the ash content of whole chicken egg with age (Senčić and Samac, 2017). The current results show that, age of hens cannot substantially affect the carbohydrate (energy) content of egg yolk but that of the albumen. Carbohydrate content of eggs is expected to be higher in younger than older birds. Therefore, eggs (especially the albumen) from younger birds can be a good source of energy for people during emergencies. Levels of fibre in egg yolk and albumen cannot be influenced by the age of chicken layers. However, fibre content of egg yolk is slightly higher than that of the albumen, which agrees with Ahn et al. (1997). The differences observed in the proximate compositions of egg components at the various hen-ages concords to the notion that, egg nutritional composition may vary due to variations in hens' age (Rizzi and Marangon, 2012). Information from the data could help in prescribing and choosing quality eggs at appropriate ages of the domestic chicken for specific people and products.

Crude protein content of egg yolk would vary with age in different chicken strains. However, the rise in the content of the nutrient from week 40 to week 53 in the Lohmann brown and White leghorn indicates the possibility of yolk protein increasing as chicken layers grow. Though albumen protein may not be affected significantly by strain-age interaction, the trait orderly increased in the three chicken strains during weeks 53, 31 and 40 accordingly. The irregular pattern of yolk and albumen protein detected shows that farmers should be guided by research findings as to when birds should be kept into lay in order to produce eggs with high protein content. However, for yolk and albumen with high protein content, eggs from the Lohmann white at weeks 40 and 53 should be considered correspondingly. Yolk fat content of domestic chickens' egg would continually decline with advancement in age as observed among the entire chicken strains against the irregular proportions of lipid found in egg yolk with advancement in layers age (Shafey, 1996). But such an interaction would not affect the albumen fat content in the layer per the current findings. This suggests that, for low fat containing eggs, chicken layers can be kept beyond week 53 with the lowest in the Lohmann white at all ages making their eggs at the periods the best choice for consumers who need small amount of fat. Nevertheless, the closely low-fat content of the albumen observed throughout the study period is an indication that, the chicken albumen produced by hens at all ages may be suitable for all classes of people and supports the low albumen fat content reported for raw egg by Roe et al. (2013). The uneven pattern of carbohydrate content of egg yolk and white from the different strain and age groups reveals possible variations in the energy levels of eggs from various chicken genotypes (strains). The energy level of yolk and albumen of the domestic chicken could be higher at week 40 in the Lohmann brown and White leghorn. The highest carbohydrate (energy) level however, should be expected in the albumen of Lohmann white at 31 weeks of age. The high level of carbohydrate in the egg white among the chicken groups makes the use of the egg component in making 'energy remedies' more viable over using the yolk.

CONCLUSIONS

Genotype (strain) and age of chicken layers can significantly affect the proximate compositions of their egg parts (yolk and albumen). For high yolk protein eggs, the birds should be kept in production up to week 53. Though albumen from all chicken strains of all ages may be good for people who need high amount of protein, the Lohmann white eggs at weeks 40 or 53 should be considered most. Yolks from commercial chickens at week 53 must be the best choice for consumers who need low fat intake. In summary, eggs must be produced from the best chicken strains and collected at the appropriate hen-ages to meet the nutritional needs of specific consumers and products. Nevertheless, the weakness of the current study is that, the results are limited to Lohmann White, Lohmann Brown and White Leghorn layer strains (genotypes) at ages 31, 40 and 53 weeks old, fed a layer mash with 18% crude protein and 3200kcal/kg ME and hence, the results cannot be generalised for other strains/breeds of different ages that are fed on different diet.

DECLARATIONS

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Availability of data and materials
Not applicable.

Authors' contributions
All authors contributed equally to this work.

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Competing interests
The authors declare that they have no competing interests.

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ADOPTING BIOSECURITY MEASURES IN CATTLE BREEDING SYSTEMS IN INDONESIA

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Supporting Information

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ABSTRACT: The study aimed to analyze the barriers to implementing biosecurity measures. Biosecurity refers to a collection of procedures designed to keep disease from spreading beyond the farm. Isolation, sanitation, and cage traffic control are all biosecurity strategies. At 2021, this study was carried out in Selli village, Bengo subdistrict, Bone regency, South Sulawesi province. Bone Regency is noted for being the largest livestock supplier in the province of South Sulawesi. A total of 35 people were chosen to be observed and questioned with the help of a questionnaire. The information gathered was analyzed using descriptive statistics. The results revealed that only 2.86 percent and 28.57 percent of respondents used quarantine or provided fences or barriers as isolation or segregation methods. Farm visitors were not provided with sanitary facilities. Everyone entering or exiting the cage was not subject to traffic control. The absence of information was the first roadblock to biosecurity adoption (65.71 percent). Then came a lack of time (31.43 percent) and a hefty price (2.86 percent). Therefore, training and extension should be provided by the Animal Hsubandary Services and Private Sectors.

Keywords: Adopting, Beef, Biosecurity measures, Cattle breeding, Smallholder farmers.

INTRODUCTION

One of the agricultural subsectors, livestock, has been critical to the Indonesian sector's growth (Sirajuddin et al., 2016). Beef cattle are a common animal kept by rural farmers in practically all of Indonesia's provinces (Prasetyo et al., 2020). The Indonesian government, corporate sector, and beef cattle farmers all supported the development of the beef cattle industry, according to Lestari et al. (2017); however, most production in Indonesia was low and did not fulfill domestic demand. This is because the bulk of beef cattle farmers has traditionally been small-scale. Beef cattle ranchers keep cattle purely as an investment that can be sold at any time for academic or religious purposes. According to Agus and Widi (2018), domestic beef supply is not keeping up with increased meat demand, accounting for less than 60% of reasonable consumption. The supply and demand for beef are growing apart. In the short-medium term, importing beef cattle and frozen meat was a quick cure. Indonesia is forced to import beef and meat from nations such as Australia. Indonesia is Australia's largest export market for cattle and beef offal, and the sixth largest market for packed meat, according to Anonymous (2020). In 2020, Australia's total export of lean meat and livestock to Indonesia was a $1.2 billion, representing 6% of total exports (Anonymous, 2020).

Every animal and public health plan, disease prevention, and control strategy must include biosecurity (Renaults et al., 2021). Biosecurity is a government policy that uses sanitation, isolation, and traffic control methods to prevent disease transmission outside or inside the cage (Anonymous, 2014; Putra et al. 2021). However, some barriers to biosecurity adoption differ significantly depending on the area, farmer socioeconomic level, and local norms. Biosecurity definitions usually include general observations about how biosecurity risks on farms should be addressed and mitigated, according to Maye and Chan (2020). On the other hand, biosecurity strategies used on farms are unevenly distributed across social groups, geographic scales, and agricultural commodity chains (Maye and Chan, 2020).

The reasons for implementing biosecurity software are to ensure that the farm is free of positive illnesses, to ensure customer threats to the products produced, to protect the framework of life and to ensure the sustainability of economic enterprises. That, and the risk of zoonotic diseases, especially for staff (Swacita, 2017). Research on biosecurity practices in cattle breeding has been done by some researchers, such as Gunn et al. (2010), who said that the ability or willingness of clients to invest in biosecurity measures was seen by veterinarians as a significant barrier. Veterinarians also believed that more efficacy and/or better evidence of the potential economic benefits of proposed farm biosecurity techniques were needed. McCarthy et al. (2021) found that protocols for quarantining visitors, colostrum feeding techniques, and calving area hygiene all had room for improvement. According to Victor et al. (2018), farmers who were identified had lax attitudes toward biosecurity under the circumstances, including the necessity to grow the size of the herd, the herd's placement in an area that was purportedly less susceptible to illness, and values associated to maintaining an

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unrestricted farming system. Other potential barriers to adopting proper biosecurity were poor communication between farmers and their workers and visitors, ignorance of infection paths, and financial restraints. Adler et al. (2019) found that the impact of personality and attitude on outcomes was significant. The personality and attitudes of farmers were associated with the health, welfare, productivity, and management of dairy cattle. Damiaans et al. (2020), found that "health management" was especially lacking in the three farm types for internal biosecurity. However, there was little discussion about the constraints of adopting biosecurity measures.

The province of South Sulawesi is one of the beef producers. After East Java and Central Java, Bone Regency is the top cattle producer in the province of South Sulawesi and third in Indonesia. The cattle population in the Bone regency is 415,073 heads, according to statistics from the Statistical Center Bureau (2020). The Indonesian government has released animal health regulations such as biosecurity applications to protect cattle from some diseases. However, not all cattle farmers adopt this regulation. Therefore it was important to conduct this research.

MATERIALS AND METHODS

This research was conducted in Selli village, Bengo subdistrict, Bone regency, South Sulawesi Province, 2021. The population is all beef cattle breeders. The sample was determined by purposive sampling with a total of 35 farmers. The data consists of primary data and secondary data. Primary data is in the form of breeder characteristics, namely gender, age, education level, number of dependents in the family, number of livestock, farming experience, land area, and distance from house to cage. Another data is the livestock business. In addition, biosecurity measures were obtained from questionnaires submitted to farmers, consisting of sanitation, isolation and traffic control. Questions on biosecurity practices are based on simple techniques that smallholder farmers can carry out. The isolation variable is quarantine (separation) for newly purchased cattle and the use of separator boundaries. The sanitation variable includes checking visitors to the cage using a foot bath, and the third variable is traffic control which consists of the management of humans, vehicles and other animals entering and leaving the cowshed area. Meanwhile, secondary data were obtained from Bone District Livestock Services and other reports. The data obtained were processed by software SPSS version 23 and analyzed descriptively using mean and percentage.

RESULTS AND DISCUSSION

Farmers' and the farm's characteristics

Table 1 shows that most respondents are male (94.29%). It is undeniable that caring for cows requires much energy, such as bathing, grazing, cleaning cages, feeding and drinking. This work is physically stronger than women, so it suits men. Looking at the respondents' ages, 94.29% belonged to the working-age category. At this age, human energy and mind are excellent. This means they are still physically strong in doing activities at home. This is so that they can raise cattle as much as possible. According to BKKBN (National Family Planning, Welfare and Population Agency), the working age category is between 15 and 64 years old.

According to the level of education, most respondents graduated from Senior High School (45.71%). This means that they have a good education. Highly educated people can better understand the new technology provided than those with low education. It can be said that they are more open-minded. The results of this study are better than that of Lambertz et al. (2012), who argued that cattle farmers' education level in Thailand was mainly graduated from primary school. Most respondents have more than 10 years of agricultural experience (51.43%), meaning they can raise cattle. Her livestock breeding experience comes from her parents. The majority of respondents were farmers (82.83%). Rice cultivation is the main source of income, and livestock breeding is a side business. Looking at the number of families, respondents, like most families, belong to the small family category 4 or more (77.14%). Therefore, the results of this study differ from Thai breeders, who have an average family size of 4.4 (Lambertz et al. 2012).

Herd size was dominated by a herd of 5-10 for each respondent (57.14%). They raise cattle semi-intensively. That is, after returning home, the cows are released into the yard in the morning and surrounded in the afternoon. The cage is made of wood or bamboo. The location of the cage is usually next to or under the house, and the distance is less than 250m. The reason for keeping cows near the house is the factor of safety from thieves.

Biosecurity practices

The Food and Agriculture Organization (FAO, 2010), claims the three main components of biosecurity are as follows: a). The process of establishing and maintaining barriers to keep infectious animals and hazardous materials out of an uninfected area is known as segregation. If done correctly, this process will prevent the bulk of contamination and infection. This includes measures such as requiring all people crossing the barrier to remove their shoes and clothing before proceeding and restricting vehicle entry; b) Cleaning: All materials entering (or leaving) a site must be thoroughly cleaned to eliminate visible filth. The majority of microorganisms that contaminate the items will also be eliminated. This indicates that there should be no visible dirt on the materials' surface. Soap, water, and a brush can be used to clean small items; c). Disinfection: Properly performed disinfection nullifies pathogens present in materials that have already been completely cleaned. Therefore, despite its importance, disinfection is the least effective biosecurity measure. Biosecurity practices are essential to be applied to the farm to prevent disease transmission. Biosecurity practices consist

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of isolation, hygiene and traffic control. Table 2 shows a simple application for biosecurity. Based on the table, it can be seen that the isolation or segregation measures, such as quarantine and the provision of fences or barriers, are carried out by 2.86% and 28.57% of respondents, respectively. The sanitation measures for farm visitors are not being put in place (100%). There is no foot bath and boot available at the entrance to the cage. The cowshed can be a source of harmful germs, either outside the building or the cows themselves. Germs from the cows can also be spread out into the surrounding area. If good sanitation facilities are not available, this can happen.

According to Dairymple and Innes (2021), all visitors must understand the risks they face when entering a farm, what farmers expect of them and what precautions are taken between the farms they visit. This applies not only to the property of goods of the same type or type but also to those who enter and leave the site and visit the property of other animals. As shown in Table 2. No traffic control exists for anyone entering or leaving the cage (100%). Everyone or other animals, such as chickens and birds, can walk in and out of the cowshed.

Table 1 - Farmers' and the farms characteristics

Variables		Mean	Frequency (person)	Percentage (%)
Sex	Male Female		33 2	94.29 5.71
Age	Unproductive Productive	47.60 ± 10.83	2 33	5.71 94.29
Education level	Primary school Intermediate school Senior high school Bachelor		6 11 16 2	17.14 31.43 45.71 5.71
Farm experience (years)	< 5 5-10 >10	11.94 ± 7.98	4 13 18	11.43 37.14 51.43
Job	Housewife Government employee Farmer Businessman		1 1 29 4	2.86 2.86 82.83 11.43
Family size	≤ 4 > 4	3.34 ± 1.77	27 8	77.14 22.86
Land size (ha)	0.25 > 0.25	0.22 ± 0.08	22 13	62.86 37.14
Herd size (head)	< 5 5 – 10 > 10	5.83 ± 2.54	13 20 2	37.14 57.14 5.71

Table 2 - Biosecurity practices in cattle breeding systems

Variables	Frequency		Percentage	
	Yes	No	Yes	No
Isolation • Quarantine • Biosecurity land boundary	 1 10	 34 25	 2.86 28.57	 97.14 71.43
Sanitation • Cleaning visitors	0	35	0	100
Traffic control • Control visitors	0	35	0	100

Adopting biosecurity measures in cattle breeding systems

According to Ritter et al. (2017), social-psychological factors are important in the adoption of farmers recommended management strategies, and disease prevention and management measures should be based on the theoretical framework that takes into account these factors. The specific situations of farmers affect the decision to adopt management strategies. The recognition of their problems and the recognized effectiveness and implementation of the recommended strategies play an essential role in determining farmers switch. The extent of the impact of internal factors on farmers (e.g. A sense of responsibility, pride or perceived social pressure) and their perceived behavioral control is evidence of their importance. Farmers are not an isolated group. They are context-sensitive and can make changes in agriculture more difficult or easier. Various advisory tools should provide consistent, accessible, relevant and feasible information to inform farmers about recommended controls. In particular, more personal means of communication can go beyond simple education to account for the farmer's individual beliefs, objectives and constraints.

Based on Table 3, it can be seen that the biggest barriers to biosecurity adoption were lack of information (65.71%), less time (31.43%) and high cost (2.86%). The study's findings were supported by Lestari et al. (2018). The top factors

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preventing beef cattle farmers from implementing biosecurity in Luwu regency, South Sulawesi province, were the shortage of extension personnel, veterinarian shortages, lack of technical knowledge of animal husbandry, and lack of finance. Lack of information lead to a lack of knowledge about animal health, which can be attributed to several factors, including lack of socialization and counseling, shortage of extension staff, remote locations and lack of financing for farming. In addition, farmers are often passive in their search for information, and they do not seek out information on their own. According to Brennan and Christley (2013), most producers thought some of the nominated biosecurity practices were valuable, but there was not always agreement between the utility of practice and its implementation. This research was found by Channanppagouda et al. (2016), a researcher argued that dairy farmers' lack of knowledge about cattle diseases and their control was a major barrier to implementing scientific animal breeding and health care procedures.

The farmers are very busy because they have to go to the fields early in the morning and come back home after the afternoon. Therefore, they have no time to bathe the cow every day; even cow dung may accumulate in the stables without cleaning. Furthermore, the high price reflects the high costs of biosecurity, such as the need to spray cages, boots, foot baths, cage clothes, and cages with a fence. This research was found by Ahmed et al. (2016), who found that one of the constraints of cattle fattening practices in urban and peri-urban kebeles of Dessie Town, Ethiopia, was the capital problem.

Table 3 - Adopting biosecurity measures in cattle breeding systems

Obstacles	Frequency (person)	Percentage (%)	Rank
Lack of information	23	65.71	I
No time	11	31.43	II
High cost	1	2.86	III

CONCLUSION

Based on the results of the study, it can be concluded that the barriers to the adoption of biosecurity measures in cattle breeding were lack of information (65.71%), no time (31.43%) and high costs (2.86 %). It is suggested that biosecurity measures can be provided to beef cattle farmers through guidance and counseling from the government and the private sector, as well as participation in biosecurity measures.

DECLARATIONS

Ethics

I declared that this research was funded under contract number: 752/UN4.22/PT.01.03.2021, 26 March 2021.

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Authors’ contribution

V Sri Lestari led and fully managed the research project and was responsible for data collection and script writing; DP Rahardja contributed to data processing and interpreting field data; SN Sirajuddin contributed to the data processing and provision of library resources; and AR Altawaha contributed to the article writing process, publication process and translation process.

Conflict of interests

None.

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PROFITABILITY OF DIETS, NUTRITIVE VALUE, PERFORMANCE AND CECAL ACTIVITY OF GROWING RABBITS FED BEAN VEIN HAY

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Supporting Information

RESEARCH ARTICLE
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ABSTRACT: A feeding trail was conducted to study effect of bean viens hay (BVH) on the growing rabbit's performance, nutritive value and cecum activity and economic return of experimental diets. Seventy two New Zealand White (NZW) rabbits (6 week of age) were divided into 6 groups and were fed 6 experimental diets inclusion BVH and Galzym® enzyme contains cellulase, xylanase, lipase, amylase, protease, pectinase, arabinase, phytase, α-galactosidase, and β-glucosidase additives. Group 1 fed negative control (basal diet without both BVH and Galzym®) and group 2 fed positive control diets (basal diet without BVH and with Galzym®). The 3rd and 4th groups fed 25% BVH substitution of clover hay without Galzym® (T3) and with Galzym® (T4). The 5th and 6th group's rabbits fed 50% BVH substitution of clover hay without Galzym® (T5) and with Galzym® (T6). The crude fiber, NDF, ADF, ADL and cellulose were higher in BVH than those in clover hay while, CP% and digestible energy (Kcal/kg) were lower in BVH than those in clover hay. Results of interaction between BVH and Galzym® additives (treatment effect) had significant ($P<0.05$) effect on rabbits productive performance, all nutrients digestibility except EE and DCP%, blood biochemical (TP, albumin, globulin, albumin/globulin ratio, cholesterol, ALT, and urea), and cecum activity (TVFA's and NH_3). Moreover, main effect of BVH was significantly ($P<0.05$) improved of rabbits productive performance, nutrients digestibility (DM, OM, CP, CF, and NFE%), blood constituents (TP, albumin, globulin, and ALT), and TVFA's in cecum. Enzyme main effect had significant ($P<0.05$) effect on FI and formation of NH_3 in rabbit's cecum. In conclusion, the dietary BVH improved the productive performance of growing rabbits without negative effect on health status. Enzyme addition increases the BVH utilization and diets profitability.

Keywords: Bean vine hay, Cecum activity, Cost, Digestibility, Rabbit.

Abbreviation: BVH: bean veins hay; FBW; final body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract; DCP: digestible crude protein; TDN: total digestible protein; DE: digestible energy; TP: total protein; alb. Albumin; ALT: aspartate aminotransferase; AST: alanine aminotransferase; TVF's: total volatile fatty acids; g: gram; mg: milligram; U: unit; dl: deciliter; NH_3: ammonia.

INTRODUCTION

Alfalfa hay considers the best fiber source use in rabbit diets that incorporated in to diets up to 40% (Gidenne et al., 2017). One of perfect solution to reduce the cost is untraditional feedstuffs (Molina et al., 2018) and wide global orientation to reduce the feed cost of feeding programs (Gidenne et al., 2017). Using the agricultural by-products in rabbit formulation diets lead to reduction of nutritional cost without any adverse effect on productive performance (Mennani et al., 2017). The chemical composition of legumes hay is close to clover hay (Feedipedia, 2016).

The nutritional value of leguminous proteins may be limited due to anti-nutritional factors (Nalle, 2009). Legumes is a good source of starch, protein dietary fiber, oligosaccharides, phytochemicals, and minerals, also, contribute to many health benefits to the human beings (Hangen and Bennink, 2002). In Egypt, the annual production of green bean (*Phaseolus vulgaris*) is estimated about 287575 ton (FAOSTAT, 2016) and bean green by-product was around 70% (Aparicio et al., 2010). A wide common bean straw (haulms) contains on DM basis about 5–11% protein, rich fiber 38–45%, 51.1-86.4% NDF, 37.3- 56.9% ADF and 5.4-9.3% lignin (Feedipedia, 2016).

The calcium and phosphorus contain were 0.68-1.15% and 0.09-0.13%, respectively. Although legumes by products are rich in protein, the nutritional value of their proteins may be limited by the presence of anti-nutritional factors (Tadele, 2015). Indigestible components presence in some feedstuffs and reduce of specific enzymes releasing from animal bodies lead to reducing the digestion about 15-25% of the diet that animals fed (Konietzny and Greiner, 2002). Enzyme supplementation to animal diets increased the nutritive value of diets due to complementary role with endogenous enzymes for young animals not mature. Also, increase availability of some nutrients for feedstuffs contains ani-nutritional factors (Cachaldora et al., 2004).

The current study aimed to investigate the influence bean veins hay as an alternative feedstuffs of clover hay in New Zealand White (NZW) growing rabbits diets on productive performance, cecum activity of growing NZW rabbits and economic profit.

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MATERIALS AND METHODS

Experiment and ethical approval

This experiment was approved under the guidelines of ethical from Cairo University, Institutional Animal Care and Use Committee (CU-IACUC, CU-II-F-6-22) and conducted in Rabbits Experimental Unit and the laboratories analyses were carried out at Department of Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Preparation of bean veins hay and diets

The cultivated fresh bean veins hay (BVH) was obtained from fields in Giza governorate, then sun-dried, and was grinded by hammer mill chemical analysis composition (AOAC, 2000) and determination of tannins (Burn, 1971), saponin (Shany et al., 1970), and phytic acid (AOAC, 2000). The ingredients were blended with BVH and pelleted in Feed Processing Unit, Faculty of Agriculture, Cairo University. A commercial enzyme named Galzym® (Tex Biosciences Ltd., India) was added to the experimental diets. The recommended dose by the producer is 0.5 g/kg diet. Galzym® contains cellulase (100000000 U), xylanase (1500000 U), lipase (10000 U), amylase (1000000 U), protease (400000 U), pectinase (30000 U), arabinase (7000 U), phytase (500000 U), α-galactosidase (10000 U), and β-glucosidase (10000 U). Six experimental diets were: T1-basal diet (negative control, NC, diet without BVH and Galzym®),T2 (positive control, PC, without BVH and with the Galzym®), T3 (25% of clover hay in NC replaced by BVH without Galzym®), T4 (25% of clover hay in NC replaced by BVH with Galzym®), T5 (50% of clover hay in NC replaced by BVH without Galzym®), T6 (50% of clover hay in NC replaced by BVH with Galzym®). All experimental diets (Table 1) were formulated to be isonitrogenous and isocaloric, to meet all the essential nutrient requirements of growing rabbits (Lebas, 2004).

Table 1 - Experimental diets formulation and chemical composition (DM% basis).

Items	Experimental diets	Control	25% BVH	50% BVH
	Without Galzym®	T1	T3	T5
	With Galzym®	T2	T4	T6
Feed ingredients				
Soybean meal (44%CP)		16.3	16.8	18.0
Yellow corn		13.8	13.8	13.8
Barley		13.0	13.0	13.0
Wheat bran		16.7	16.2	15.0
Clover hay		34.0	25.5	17.0
Bean vein hay (BVH)		0.0	8.5	17.0
Dl- methionine		0.2	0.2	0.2
Di-Cal-phos		2.2	2.2	2.2
NaCl		0.3	0.3	0.3
Min & Vit Mix1		0.3	0.3	0.3
Anti-coccidia & fungi		0.2	0.2	0.2
Molasses		3.0	3.0	3.0
Total		100	100	100
Chemical analysis (DM basis)				
Dry matter (DM, %)		82.80	83.30	83.77
Organic matter (OM, %)		85.64	84.82	84.08
Crude protein (CP, %)		17.20	17.03	17.07
Crude fiber (CF, %)		13.90	14.67	15.44
Ether extract (EE, %)		2.20	2.24	2.29
Nitrogen free extract (NFE, %)		55.34	54.35	53.23
Ash (%)		5.08	5.52	5.97
Digestible energy (DE, Kcal/kg)2		2612	2608	2612
Calcium (%)		1.00	0.99	1.02
Total phosphors (%)		0.50	0.50	0.50

[1] Commercial vitamin and mineral premix contained (per 3 Kg premix) Vit. A 12000 000 IU, Vit. D3 3000 000 IU, Vit. E 10 000 mg, Vit. K3 2000 mg, Vit. B1 1000 mg, Vit. B2 5000 mg, Vit. B6 1500mg, Vit. B12 10 mg, Pantothenic acid 10 000 mg, Nicotenic acid 30 000 mg, Folic acid 1000 mg, Biotin 75 mg, Copper 4000 mg, Manganese 80 000 mg, Zinc 50 000 mg, Iron 30 000 mg, Iodine 500 mg, Selenium 100 mg and Cobalt 100 mg. [2]DE= Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Animal and management

A total of 72 New Zealand White (NZW) rabbits aged 42 days weighing about 744.91 ± 5.27g were randomly allocated to 6 groups (12 rabbits/group). The experiment lasted 8 weeks (6-14 weeks of age). Rabbits housed in metal battery cages (30×35×40 cm) supplied with separated feeders. The animals were provided *ad-libitum* access to automatic nipple fresh water drinkers and pelleted feed throughout the experimental period. All animals were kept under the same hygienic conditions and management. Also, all rabbits were vaccinated against diseases.

Animal performance measurements

Rabbits and feed weights were taken every week. Rabbit's body weight were weighed individually and averaged by cage for statistical analysis. Mortality was recorded daily. Body weight (BW), body weight gain (BWG), feed intake (FI), mortality, and feed conversion ratio (FCR, g.feed : g.gain) were determined on a per cage basis, and then averaged by treatment.

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Digestion trial

Rabbits divided into 6 groups of 5 rabbits each for digestion trial execution (Perenz et al., 1995). Rabbits were placed in individual metabolism cages (56×38×28 cm). Feces were collected daily before the morning meal and weighed freshly and dried at 60 °C for 24 hour in air-drying oven. The BVH, experimental diets and feces were prepared to analyze moisture, ash, nitrogen, ether extract (EE), and crude fiber (CF). Data of quantities and chemical analysis (AOAC, 2000) of feed and feces were used to calculate the nutrient digestion coefficients, nutritive value (Fekete, 1985) and digestible energy (DE Kcal/kg; Schneider and Flatt, 1975) for each dietary experimental treatment.

Blood constituents

Five rabbits from each treatment were randomly selected to collect the blood samples at the end of growing period. During slaughtering time, blood samples were collected in heparinized glass tubes and centrifuged at 3000 rpm for 20 minute, then samples were transferred and stored in deep freezer at -20ºC till biochemical analysis kits assay (Purchased: Bio-diagnostic Co, Egypt). All plasma biochemical measurements; total protein (Gornall et al., 1949), albumin (Doumas and Waston, 1971), plasma globulin concentration (the difference between total protein and albumin), creatinine and urea (Folin, 1934; Tabacco et al. 1979), and aspartate aminotransferase and alanine aminotransferase (Henry, 1964) were assayed by colorimetric methods and performed according to manufacturer's instruction.

Cecum activity

All rabbits slaughtered (after 16 hour fasting) for blood constituents collection at end of 14th week also had ceca collected to determine cecum characteristics. Each sample of cecum content was strained through 4 folds of gauze and divided into 2 portions. The 1st portion was used immediately for measuring the pH value and ammonia nitrogen concentration (Conway, 1958). The 2nd portion was preserved by the addition of 1ml HCl (N/10) and 2 ml orthophosphoric acid to each 2 ml of cecum contents juice for total volatile fatty acids (TVFA's) determination (Eadie et al., 1967).

Economic profit

The calculation of economic efficiency is dependent on total cost and selling price of live body weight (2.43$) at the time of experiment execution.

Statistical analysis

Data were analyzed using general linear model SAS software, version 9.2. (SAS Institute, 2004, USA). Duncan's multiple range test was performed to detected significant differences between means when F-test is significant. The significant was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Nutritional composition and phytochemicals of bean veins hay (BVH)

The estimated chemical analysis contents (DM, and CF%) of BVH were higher than those in clover hay, while, crude protein (CP%), ether extract (EE%) and nitrogen free extract (NFE%) and digestible energy (DE kcal/kg) were lower than those in clover hay (Table 2). Organic matter (OM%) and ash (%) content of BVH were similar to clover hay. The cell wall contents (NDF, ADF, ADL, hemicellulose and cellulose) of BVH were increasing when comparable of clover hay except hemicellulose was decreasing. The mineral contents (calcium and total phosphors) in BVH were lower than those in clover hay (1.43 and 0.20% vs 1.60 and 0.35%, respectively). As the same trend, the CF and CP of BVH on DM basis ranged from 38-45% and 5-11%, respectively (Feedipedia, 2016). However, the approximate analysis for BVH were 87.78% DM, 80.43% OM, 22.37% CP, 29.00% CF, 4.37% EE, 24.69% NFE, 19.57% ash, and 2000 Kcal DE/kg. Moreover, BVH cell wall contents from NDF, ADF, ADL, hemicellulose and cellulose were 47.98, 35.88, 12.45, 12.10, and 23.43 %, respectively (Lounaouci-ouyed et al., 2014; Abou El-Fadel et al., 2019). The values of phytochemicals (phytic acid, tannins, and saponins) were 1.00 g/100g DM, 1.85g/100g DM and 1.23%, respectively (Table 2). Similarly, phytic acid content of BVH was 0.5-1% (Vasić et al., 2012) and phytic acid, tannin and saponins concentration of BVH were 1.00, 1.85, and 1.23%, respectively (Mohamed, 2020). Tannins have been positive effects on animal healthy by reducing the gastro intestinal pathologies in mammals (Min et al., 2005).

Productive performance

As presented in Table 3, there were significant differences ($P<0.05$) interaction between BVH level and Galzym® addition on final BW ($P<0.0001$) and daily BWG ($P<0.0001$). Final BW and daily BWG were significantly ($P<0.05$) increased with all groups fed dietary BVH compared to control groups, with no significant differences between 25 and 50% replacement BVH levels. Final BW and BWG significantly affect ($P<0.0001$) by substitution level of BVH, but Galzym® addition had no effect ($P=0.52$ and $P=0.50$, respectively). There were significant effect of dietary BVH levels ($P<0.0001$) and Galzym® addition ($P=0.05$) on average FI (Table 3) and a significant interaction was noted between BVH level and Galzym® addition ($P<0.0001$). Rabbits in negative and positive control groups consumed lower ($P<0.05$) than those in other groups. The rabbits fed two level substitution of BVH without enzyme consumed higher ($P<0.05$) than rabbits fed BVH with enzyme groups. The crude fiber content (Table 2) in BVH (39.70%) higher than clover hay (26.00%) due to this increase the fiber content in dietary BVH increased the consumed feed (Blas and Mateos 2020). A significant interaction effect was observed between BVH level and Galzym® addition ($P=0.05$) on FCR (Table 3). There was also significant effect of BVH levels ($P=0.03$). While, average FCR did not differ significantly ($P=0.07$) due to enzyme addition. Positive control had lower FCR value (2.24) followed by T6 (2.99) than other treatment groups, while negative control group had the highest FCR value (3.41). Nevertheless, the negative control rabbits was not significant difference in FCR with those fed 25 and 50% BVH without enzyme addition (T3 and T5).

As the same trend, rabbits fed high level of dried waste green bean at 30% were significantly ($P<0.05$) increased in FBW and BWG. While, not significant differences ($P>0.05$) were observed in consumed feed and improving FCR (Abou El-Fadel et al., 2019). The improvement in FBW, daily BWG and FI due to phytochemicals (tannins, saponins and phytic acid)

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content of bean veins hay. The tannins can protect intestinal mucosa against oxidative damage and pathogens and limit peristaltic activity in digestive disorders preventing diarrhoea (Kermauner and Lavrenčič, 2008). Using the saponins as phytochemicals feed additives in pig diets caused improving the body weight, daily weight gain and feed intake Bartoš et al. (2016). Multi-enzyme addition (Natuzym; cellulase, xylanase, β-glucanase, α-amylase, protease and lipase) not affected (P>0.05) on productive performance of cross-breeds growing rabbits (Ayodele et al., 2016).

In contrast, amylofeed® enzyme (amylase, B-glucanase and B-xylnase) affected (P<0.05) on productive performance of growing rabbits (Cachaldora et al., 2004). Nevertheless, the productive performance of growing Algerian white rabbit was not significantly difference (P>0.05) when compared rabbits fed completely replacement of protein source in a basal soybean meal diet by 26% filed bean group with rabbits fed control diet group (Lounaouci-ouyed et al., 2014). Also, final BW BWG, and FCR were not significantly differences (P>0.05) between rabbits fed different levels of bean waste up to 30% and those fed control diet (Hervé et al., 2019). Growth rabbit's performance improved (P<0.05) by enzyme addition from 25-39 days of age (Gutierrez et al., 2002), protease addition (Al-Sagheer et al., 2020), and exogenous enzyme ZAD® (a biotechnical product made from natural sources) and ZAD® combined with *Lactobacillus acidophilus* (Abdel-Aziz et al. 2014). Final BW and BWG not significantly differences (P>0.05) between broiler fed green bean up to 16% while, broilers fed green bean with Benzyme-A (commercial enzyme contains cellulase 700000 U, amylase 100000 U, pectinase 60000 U, phytase 100000 U, xylanase 1000000 U, and carrier) were significantly (P<0.05) increased final BW and BWG (Abbel-Monein, 2013). The enzyme addition did not affect growth performance parameter that may be due to lower enzyme dose and amount of substrate in experimental diets (Suliman, 2012).

Table 2 - Chemical analysis and phytochemicals of clover hay and bean vein hay

Items	Clover hay	Bean vein hay (BVH)
Dry matter (DM, %)	91.95	92.70
Organic matter (OM, %)	87.50	87.27
Crude protein (CP, %)	13.45	9.67
Crude fiber (CF, %)	26.00	39.70
Neutral detergent fiber (NDF, %)	42.50	56.85
Acid detergent fiber (ADF, %)	29.50	49.40
Acid detergent lignin (ADL, %)	5.50	12.00
Hemicelluloses%[1]	13.00	7.45
Celluloses%[2]	24.00	37.40
Ether extract (EE, %)	4.00	1.05
Nitrogen free extract (NFE, %)	44.05	36.50
Ash (%)	12.50	12.73
Digestible energy (DE, Kcal/kg)[3]	2104.74	1664.66
Calcium (%)	1.60	1.43
Total phosphors (%)	0.35	0.20
Phytochemicals		
Phytic acid (g/100 g DM)	–	1.00
Tannin (g/100 g DM)	–	1.85
Saponins (%)	–	1.23

[1]Hemicellulose% = NDF – ADF, [2]cellulose% = ADF – ADL, [3]DE (kcal/g) = 4.36 - 0.049 x [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Table 3 - Effect of tested diets on productive performance of NZW growing rabbits

Items	IBW (g)	FBW (g)	BWG (g/day/rabbit)	FI (g/day/rabbit)	FCR (gain: feed)
T1 (negative control)	746.11	2064.69[b]	23.54[b]	79.28[c]	3.41[a]
T2 (positive control)	743.89	2165.81[b]	25.39[b]	81.39[c]	2.24[b]
T3	742.78	2369.91[a]	29.06[a]	90.31[a]	3.12[ab]
T4	746.11	2334.04[a]	28.36[a]	86.33[b]	3.08[b]
T5	747.22	2335.56[a]	28.36[a]	90.73[a]	3.22[ab]
T6	742.78	2350.96[a]	28.72[a]	85.19[b]	2.99[b]
SEM	0.40	20.97	0.37	0.63	0.042
P-value					
Treatment effect	0.34	<0.0001	<0.0001	<0.0001	0.05
Level substitution of BVH	0.21	<0.0001	<0.0001	<0.0001	0.03
Galzym® additive effect	0.17	0.52	0.50	0.05	0.07

Mean values with different superscript letters in a column are significantly different (p<0.05). T1: negative control diet without BVH and Galzym®; T2: positive control diet without BVH and with the Galzym®; T3: 25% of clover hay in basal diet replaced by BVH without Galzym®; T4: 25% of clover hay in basal diet replaced by BVH with Galzym®; T5: 50% of clover hay in basal diet replaced by BVH without Galzym®; T6: 50% of clover hay in basal diet replaced by BVH with Galzym®. IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake; FCR; feed conversion ratio.

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Table 4 - Effect of tested diets on nutrients digestibility coefficients and nutritive value of tested diets									
Items	Digestion coefficients (%)						Nutritive value		
	DM	OM	CP	CF	EE	NFE	DCP%	TDN%	DE Kcal/kg
T1(negative control)	60.15[b]	63.70[c]	75.15[b]	44.92[b]	75.37	73.83[c]	13.22	65.32[c]	2893.70[c]
T2 (positive control)	64.96[a]	66.90[b]	75.69[b]	45.57[b]	75.79	78.61[ab]	13.43	68.12[ab]	3017.85[ab]
T3	66.57[a]	68.74[a]	77.79[a]	50.14[ab]	76.99	80.30[a]	13.51	68.29[a]	3025.17[a]
T4	65.89[a]	67.28[ab]	77.81[a]	51.31[a]	77.37	77.69[b]	13.40	66.81[ab]	2959.68[ab]
T5	65.09[a]	66.72[b]	76.11[b]	51.57[a]	77.05	77.61[b]	13.63	66.65[bc]	2952.74[bc]
T6	64.71[a]	66.67[b]	77.98[a]	53.50[a]	76.77	77.43[b]	13.55	67.41[ab]	2986.51[ab]
SEM	0.48	0.36	0.30	0.90	0.38	0.47	0.05	0.003	0.003
P-value									
Treatment effect	<0.0001	0.0002	0.003	0.009	0.650	0.0001	0.300	0.003	0.003
Level substitution of BVH	0.002	0.005	0.001	0.005	0.190	0.049	0.110	0.460	0.460
Galzym® additive effect	0.200	0.460	0.190	0.500	0.820	0.500	0.590	0.200	0.200

Mean values with different superscript letters in a column are significantly different (p<0.05). T1: negative control diet without BVH and Galzym®; T2: positive control diet without BVH and with the Galzym®, T3:25% of clover hay in basal diet replaced by BVH without Galzym®; T4: 25% of clover hay in basal diet replaced by BVH with Galzym®; T5: 50% of clover hay in basal diet replaced by BVH without Galzym®; T6: 50% of clover hay in basal diet replaced by BVH with Galzym®. DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fiber; EE: ether extract; NFE: nitrogen free extract; DCP: digestible crude protein; TDN: total digestible nutrients; DE: digestible energy.

Nutrients digestibility coefficients and nutritive value

As presented in Table 4, there were significant differences in digestibility coefficients of DM (P<0.0001), OM (P=0.0002), CP (P=0.003), CF (P=0.009), and NFE (P=0.0001) due to interaction between BVH level and Galzym® addition, but EE digestibility not affected (P=0.65). The negative control group (T1) was significantly (P<0.05) decreased in DM%, OM%, and NFE% digestibilities in comparison with other experimental groups. The CP% and CF% digestibility coefficients improved (P<0.05) with different levels of BVH except CP% of T5 not improved. The NFE% digestion coefficient recorded best value (P<0.05) with T3 group compared with all experimental groups except positive control group. The effect of BVH level substitution did statistically (P<0.05) affected on all nutrients digestion except EE digestion was not effect, while enzyme addition was not effect (P>0.05). The inclusion of dietary filed bean in rabbits not affected (P>0.05) on nutrient digestion coefficient (Lounaouci-ouyed et al., 2014). For nutritive value (Table 4), there were significant interaction between BVH level and enzyme addition on total digestible nutrients (TDN%; P=0.003) and digestible energy (DEKcal/kg; P=0.003), while digestible crude protein (DCP%) not affected significantly (P=0.300). The TDN% and DE were significantly (P<0.05) improved with all trial groups when compared to negative control group (T1) except T5 recoded not significantly. The main effects of BVH levels substitution and enzyme addition did not effect on nutritive value.

Similar results were reported for digestibility of DM, OM, NDF and ADF% of growing rabbits did not affected (P>0.05) by Natuzyme addition (Ayodele et al., 2016). However, the nutrients digestibility and nutritive value (DCP%, TDN% and DE kcal/kg) improved by protease addition to growing rabbit diets (Al-Sagheer et al., 2020) Also, nutritive value of nutrients by enzyme addition (ZAD®) and mixed it with *Lactobacillus acidophilus* (Abdel-Aziz et al., 2014). The nutrients digestion coefficients of dietary dried green bean and nutritive value (DCP% and DE kcal/kg) were significantly (P<0.05) differences (Abou El-Fadel et al., 2019). The improvement of nutrients digestion and nutritive value for BVH dietary may be due to phytochemicals content (Tannins, saponnin and phytic acid) that have been antioxidant property (Dost and Tokul, 2005). It was enhanced by protecting against oxidation and improving nutritional value (Corino and Rossi, 2021).

Plasma constituents

As presented in Table 5, the effect of interaction between BVH level and Galzym® addition were affected on total protein (TP g/dl; P=0.003), albumin (g/dl; P=0.0001), globulin (g/dl; P=0.030), albumin/globulin ratio (P=0.0005), cholesterol (mg/dl P=0.010), ALT (U/l; P=0.0001), and urea (mg/dl; P=0.006). While, no significant interaction was found regarding to AST (U/l; P=0.120) and creatinine (mg/dl; P=0.090). The TP and albumin increased (P<0.05) with T5 when compared to all tested groups included the control groups. The rabbits fed T6 and T5 diets gave the highest concentration of globulin (2.60 g/dl) and albumin/ globulin ratio (1.90), respectively. The lowest value (P<0.05) of cholesterol (100 mg/dl) had recorded with rabbits fed positive control diet (T2). The ALT concentration was significantly (P<0.05) decreased with different levels of dietary BVH groups (without or with Galzym®) compared to negative or positive control groups. The urea concentration was lowest (P<0.05) with T3 (20.50mg/dl) and T6 (20.50mg/dl) groups. Moreover, there were significant differences in total protein (P=0.005), albumin (P=0.030), globulin (P=0.003), and ALT (P=0.0001) due to main effect of BVH levels substitution. Abou El-Fadel et al. (2019) observed that the rabbits fed different level of dried green bean up to 30% had significantly differences in albumin, ALT, urea, total cholesterol. However, the alternative crude fiber source caused significant effect on urea, AST and cholesterol due to lipids metabolism in growing rabbits (Petkova et al., 2011). The main effect of Galzym® addition had not effect (P>0.05) on all plasma parameters except albumin/globulin (P=0.030). As same trend, TP, albumin, glubulin, cholesterol, ALT, and AST not influence (P>0.05) by multi-enzyme addition (xylanase, cellulose, beta-glucanase, pectinase, amylase, protease, lipase, phytase, glactosidase, and mannanase) in growing rabbit diets (Sherif, 2018). Moreover, Abd El-Ghani et al. (2018) found that the enzyme additive significantly affect (P<0.05) on AST and AST/ALT ratio concentration of growing rabbit blood.

Cecum activity

As presented in Table 6, there were significant interactions between BVH level and enzyme addition on TVFA's (mleq./100ml; P=0.0001) and NH_3 (mg/100ml; P=0.0001), however, no interaction effect on pH (P=0.640) in cecum contents. Little effect showed in pH value with rabbits fed all dietary BVH (P=0.370) and enzyme addition (P=0.260). The

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TVFA's was significantly (P<0.05) increased with rabbits fed dietary BVH without or with enzyme compared to those in control groups. The NH_3 was significantly (P<0.05) increased in cecum for groups fed all diets contain enzymes compared the groups fed diets without enzyme including positive control group. Probability value had significant with main effect of BVH levels substitution for TVFA's (P=0.0001), while NH_3 was not affected (P=0.620). Main effect of enzyme addition had not affected on TVFA's (P=0.900), while NH_3 had significant (P=0.0001). The improved in cecum activity explained by Petkova et al. (2011) found that alternative crude fiber sources, such as meadow hay or straw make caused a best environmental condition for benefit microbes growth in cecum which lead to better fermentation. Also, enzymes additives enhanced on microflora growth in rabbit's gut and cecum caused increasing in volatile fatty acids (VFA's) production (Abd El-Latif et al., 2008).

Economic profit

Data concerning of profitability and economic efficiency of trial diets are shown in Table 7. According the results of performance (Table 3) showed improving in FBW (g) of rabbits fed all diets contains BVH. Also, FCR improved with enzyme addition diets. The economic efficiency increased with groups fed enzyme diets including the positive control group (T2) also, this improving increased with the several levels of BVH. The net revenue and economic efficiency improved with BVH dietary without or with exogenous enzymes. The best economic efficiency observed with T6 group followed by T4, T3 and T5. Similarly, the economic profit increased with feeding growing rabbit on diets containing peanut veins hay diets (Omer et al., 2017). The economic efficiency of dietary dried waste green bean also increased due to increase the level of dried waste green bean (Abou El-Fadel et al., 2019). Inclusion of dietary bean offal in growing rabbit reduced the total feed cost (Hervé et al., 2019).

Table 5 - Effect of tested diets on blood measurements of growing NZW rabbits

Items	TP (g/dl)	Alb. (g/dl)	Globulin (g/dl)	Al/G ratio	Cholesterol (mg/dl)	Liver function		Kidneys function	
						ALT (U/l)	AST (U/l)	Creatinine (mg/dl)	Urea (mg/dl)
T1 (negative control)	6.70^{cb}	4.30^{b}	2.40^{b}	1.79^{bc}	110.00^{a}	34.05^{a}	12.17	1.00	21.82^{a}
T2 (positive control)	6.35^{c}	4.00^{c}	2.35^{b}	1.70^{cd}	100.00^{b}	29.40^{b}	11.54	0.95	22.50^{a}
T3	6.65^{bc}	4.30^{b}	2.35^{b}	1.83^{ab}	108.00^{a}	24.30^{c}	11.09	0.95	20.50^{b}
T4	6.60^{bc}	4.30^{b}	2.30^{b}	1.87^{ab}	107.50^{a}	24.20^{c}	11.75	1.20	22.17^{a}
T5	7.25^{a}	4.75^{a}	2.50^{ab}	1.90^{a}	112.50^{a}	24.25^{c}	11.78	1.00	21.67^{a}
T6	6.85^{b}	4.25^{b}	2.60^{a}	1.64^{d}	112.50^{a}	24.25^{c}	12.52	1.00	20.50^{b}
SEM	0.078	0.059	0.032	0.0005	1.25	0.90	0.159	0.29	0.22
P-value									
Treatment effect	0.003	0.0001	0.030	0.0005	0.010	0.0001	0.120	0.090	0.006
Level substitution of BVH	0.005	0.030	0.003	0.210	0.370	0.0001	0.180	0.370	0.110
Galzym® additive effect	0.080	0.200	0.990	0.030	0.170	0.610	0.420	0.260	0.390

Mean values with different superscript letters in a column are significantly different (p<0.05). TP: total protein; Alb.: albumin; Al/G: albumin globulin ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase; T1: negative control diet without BVH and Galzym®; T2:positive control diet without BVH and with the Galzym®, T3:25% of clover hay in basal diet replaced by BVH without Galzym®; T4: 25% of clover hay in basal diet replaced by BVH with Galzym®;T5: 50% of clover hay in basal diet replaced by BVH without Galzym®; T6: 50% of clover hay in basal diet replaced by BVH with Galzym®.

Table 6 - Effect of tested diets on cecum activity of growing NZW rabbits

Items	pH	TVFA's (mleq./100 ml)	NH_3 (mg/100 ml)
T1(negative control)	5.94	5.23^{c}	6.84^{b}
T2 (positive control)	6.00	5.30^{c}	9.37^{a}
T3	6.87	6.17^{b}	7.55^{b}
T4	5.97	6.40^{b}	9.92^{a}
T5	6.00	6.80^{a}	7.70^{b}
T6	6.03	6.40^{b}	9.15^{a}
SEM	0.03	0.13	0.26
P-value			
Treatment effect	0.64	0.0001	0.0001
Level substitution of BVH	0.37	0.0001	0.62
Galzym® additive effect	0.26	0.90	0.0001

Mean values with different superscript letters in a column are significantly different (p<0.05). TVFA's: total volatile fatty acids; NH_3: ammonia production; T1: negative control diet without BVH and Galzym®; T2: positive control diet without BVH and with the Galzym®, T3:25% of clover hay in basal diet replaced by BVH without Galzym®; T4: 25% of clover hay in basal diet replaced by BVH with Galzym®; T5: 50% of clover hay in basal diet replaced by BVH without Galzym®; T6: 50% of clover hay in basal diet replaced by BVH with Galzym®.

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Table 7 - Effect of tested diets on economic profit

Items \ Experimental treatments	T1	T2	T3	T4	T5	T6
Total average weight (kg)	1.32	1.42	1.63	1.59	1.59	1.61
Price of one kg body weight ($)	2.43	2.43	2.43	2.43	2.43	2.43
Selling price/rabbit ($)(A)	3.21	3.46	3.96	3.86	3.86	3.91
Total feed intake (kg)	4.44	4.56	5.06	4.83	5.08	4.77
Price/kg feed($)	0.23	0.23	0.23	0.23	0.23	0.23
Total feed cost/rabbit ($)(B)	1.02	1.06	1.15	1.11	1.16	1.10
Net revenue ($)[1]	2.19	2.40	2.80	2.75	2.70	2.81
Economic efficiency[2]	2.14	2.27	2.43	2.47	2.33	2.56

[1]Net revenue: A – B; [2]Economical efficiency (%): (Net revenue / B) × 100. T1: positive control diet without BVH and Galzym®; T2: negative control diet without BVH and with the Galzym®, T3: 25% of clover hay in basal diet replaced by BVH without Galzym®; T4: 25% of clover hay in basal diet replaced by BVH with Galzym®; T5: 50% of clover hay in basal diet replaced by BVH without Galzym®; T6: 50% of clover hay in basal diet replaced by BVH with Galzym®. LE= 0.064$

CONCLUSION

It could be concluded that BVH can be fed the New Zealand White growing rabbits from 6 to 14 week of age at 25% and 50% substitution of clover hay in basal control diet without adverse effects on performance, health status, blood biochemical parameters, and both liver and kidney functions.Bean veins hay increased the market weight of growing rabbits and improved the nutritive value. Also, bean veins hay with Galzym® enzyme addition can improve feed conversion ratio. Enzyme addition was effectively in protein and fiber digestion and ammonia production in cecum by microflora. Enzyme addition had positive effect on cost return of diets.

DECLARATIONS

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Authors' contribution

Dr. M.A. Suliman designed the experiment, and drafted the manuscript. Dr. D.M. Saber performed the practical part, collaborated the chemical analyses and the statistical analysis, tabulation of the experimental data. Dr. M.A. Manylawi and Dr. M.R. Ibrahim collaborated in the main idea and participated in manuscript review.

Conflict of interests

The authors have declared that no competing interest exists.

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BULL SPERM AND SEMINAL PLASMA PROTEINS AND THEIR RELATIONSHIP WITH FERTILITY: A REVIEW

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Supporting Information

ABSTRACT: The efficiency of artificial insemination (AI) is greatly influenced by the quality of semen. Sperm and seminal plasma are found in semen, which play a role in the reproductive process and its ability to fertilize an egg and maintain the development of an embryo. Various factors will determine the fertility capacity of a sperm, both from the intrinsic factors of the sperm and the plasma component of the semen. Seminal plasma proteins are crucial for maintaining the stability of the membrane, viability, motility of sperm, acrosome reactions, maintaining osmotic pressure and helping the fertilization process. Good quality semen will support the fertilization process. The purpose of this scoping review is to increase our understanding of protein from sperm and seminal plasma of bulls and their relationship with fertility. The sperm proteins that were significantly correlated with fertility were Outer Dense Fiber protein 2 (ODF2), Protamine (PRM), Testis specific histone 2B (TH2B), Phosphatidylethanolamine binding protein (PEBP4), and Ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRSC2). Meanwhile, the seminal plasma proteins positively correlated with fertility were Osteopontin (OPN), Phospholipasea 2 (PLA2), P25b, Acidic seminal fluid proteins (aSFP), Alpha-L-fucosidase (α-L-fucosidase), and Bovine seminal plasma (BSP).

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INTRODUCTION

Bull fertility determines the success of pregnancy in females and is an important factor in the sustainability of livestock. Bull fertility can be measured based on the ability of sperm to fertilize oocytes, reproductive efficiency (Kaya and Memili, 2016), pregnancy, number of births, non-return rate, pregnancy rate (Berry et al., 2014), libido, and testosterone hormone concentration (Iskandar et al., 2022). Bull fertility was phenotypically performed using the Breeding Soundness Examination (BSE) method at the AI center (Butler et al., 2020). A bull is assessed according to the BSE standards in three categories, including physical scrotal circumference, sperm progressive motility, and sperm morphology (Ugur et al., 2022). Molecularly, bull fertility can be carried out using proteomic analysis. It is a study to analyze how the molecular processes of sperm function are related to fertility (Aitken and Baker, 2008; Olivia et al., 2009).

Sperm contains proteins that support metabolic processes and help in redox regulation of cells (Barranco et al., 2019; Gaitskell-Phillips et al., 2020; Gaitskell-Phillips et al., 2020; Pena et al., 2021). Proteins are biomolecules that can be found in cells. Seminal plasma proteins interact with sperm transported in microvesicles; as a result, these proteins can affect and regulate sperm activity (Rodriguez-Martinez et al., 2021). Sperm proteins play a role in embryo growth and successful fertilization (McReynolds et al., 2014). Sperm are highly specialized and transcriptionally active cells; protein cascades may be involved in inducing sperm motility (Siva et al., 2010). The availability of sperm energy sources from seminal plasma in the form of fructose, sorbitol, plasmogen, and glyceryphosporil choline can also affect sperm motility (Sundari et al., 2013). Sperm and seminal plasma are sources for investigating bull fertility. Bull fertility has been defined as sperm's ability to fertilize oocytes and embryonic development (Kaya and Memili, 2016), as well as improvements in cattle genetic selection (Viana et al., 2018).

The seminal plasma of semen consists of various specific biochemical components that regulate the functions of sperm. Components in seminal plasma have functions in sperm cells and in the female reproductive tract (de Andrade et al., 2012; Caballero et al., 2012; Rodriguez-martinez et al., 2021). Seminal plasma has been proven to have a helpful process for sperm and fertility, increasing longevity, and sperm mobility within the female ducts (Druart et al., 2019). The main components of seminal plasma are water and both organic and inorganic materials. The seminal components of plasma consist of ions, energy substrates (mainly fructose in the case of male sperm), organic compounds, peptides, and proteins. The seminal plasma components play a crucial role in the fertilization process, motility, capacitation, and interaction of egg and sperm cells (Juyena and Stalletta, 2012). The seminal plasma protein has been investigated

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previously, which is found to be a molecular marker in different species related to fertility. The majority of the investigation into the characterization of these proteins has been reported on boar, bull, buck, ram, stallion, and poultry (Jonakova et al., 2010). Seminal plasma has functions in membrane stabilization, sperm viability, the process of capacitation reactions, acrosome reactions, and fertilization (Barrios et al., 2000). The association between sperm and seminal plasma proteins of bull and fertility will be covered in this review. Conclusively, the proteins of sperm and seminal plasma of bulls can provide information and understand functions related to fertility.

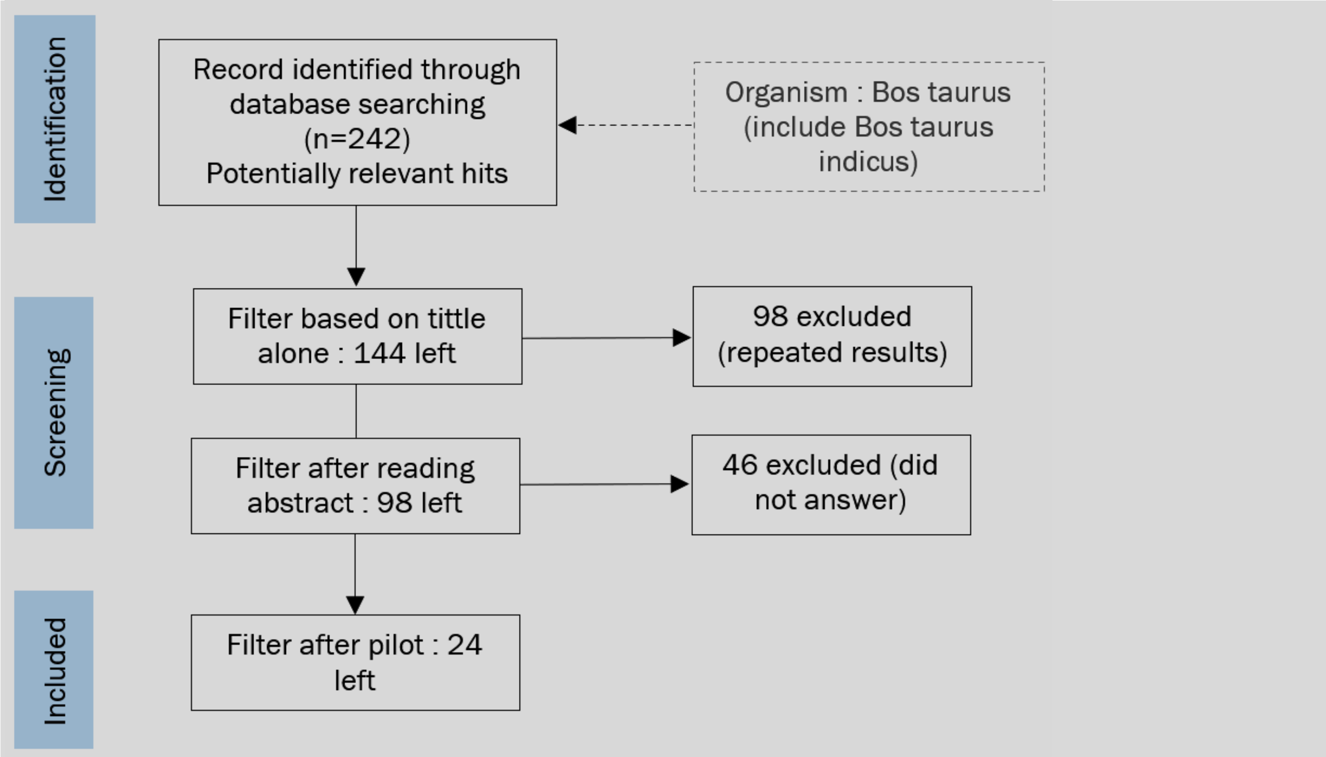

Figure 1 - Systematic review according to the protocol preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Sperm chromatin dynamics

The plasma membrane, flagellum, cytoplasm, acrosome, and nucleus of the sperm include a variety of proteins that are important to the physiology of the sperm (Rawe et al., 2008). Chromatin is the physiological substrate for genetic processes in the nucleus of eukaryotic cells. Dynamic changes in chromatin emerge as key regulators of genomic function (Fischle et al., 2003). During the transition from spermatogonia to spermatozoa, there are histone changes (Figure 1). The complex process of spermatogenesis occurs in three stages. The first stage involves the process of mitotic cell division which allows the initial stage of the cell, the spermatogonia, to reproduce. The second stage of meiosis, in which diploid cells form haploid cells, is the process of division until the formation of spermatids. The final stage of spermatogenesis includes the production of sperm, mature, and motile sperm cells, through the process of spermiogenesis (de Kretser et al., 1998).

Spermatogenesis is the process of producing functionally mature sperm from precursor sperm (Hao et al., 2019). Lysine and cysteine residues in protamine are distinct from those in core histones. Chromatin condensation and the substitution of protamines for histones occur in conjunction with the last stages of spermatogenesis (Sahoo et al., 2021). The nucleohistone complex, which contains histone H2A, H2B, histone 3 (H3), histone 4 (H4), and protamines (PRM), is firmly coiled around sperm DNA (Miller et al., 2010). Transition proteins 1 (TP1) and 2 (TP2) replace core histones during spermatogenesis, and transition proteins (TPS) are replaced by testis-specific protamines (Zhao et al., 2001; Balhorn, 2007). One of the most important phases in sperm chromatin remodeling is histone-to-protamine exchange, because it controls the degree of chromatin condensation, which is required for fertilization (Ugur et al., 2019). Round spermatids' chromatin structure and cellular shape undergo substantial changes throughout spermiogenesis. It results in sperm to have severely compacted chromatin and to experience transcriptional quiescence (Özbek et al., 2021).

Sperm chromatin during spermatogenesis is mostly packed with protamine (85-98%), forming toroid DNA (Kutchy et al., 2017). Histone-bound DNA loci encodes transcription and takes a pivotal part during postfertilization and early embryonic development (Hammoud et al., 2009). Sperm and chromatin are packaged efficiently, because the replacement of histones with protamine is aimed at egg fertilization, egg activation, and embryonic development (Balhorn et al., 1988). The association of protamines with DNA brings about a unique chromatin remodeling in sperm and appears to facilitate the hydrodynamic shape of the sperm head (Brunner et al., 2014). Sperm chromatin integrity is critical for successful fertilization, healthy egg activation, embryo development, and species survival (Gawecka et al.,

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2013). Advanced techniques are required to evaluate the health of sperm chromatin, since it has such a significant influence on the bull's ability to reproduce (Kutchy et al., 2017). Linker histones are progressively replaced by testis-specific variations during spermatogenesis, and histones are replaced by transition proteins, protamines, and finally protamine-like substances (sperm chromatin health) (Kimmins and Sassone-Corse, 2005).

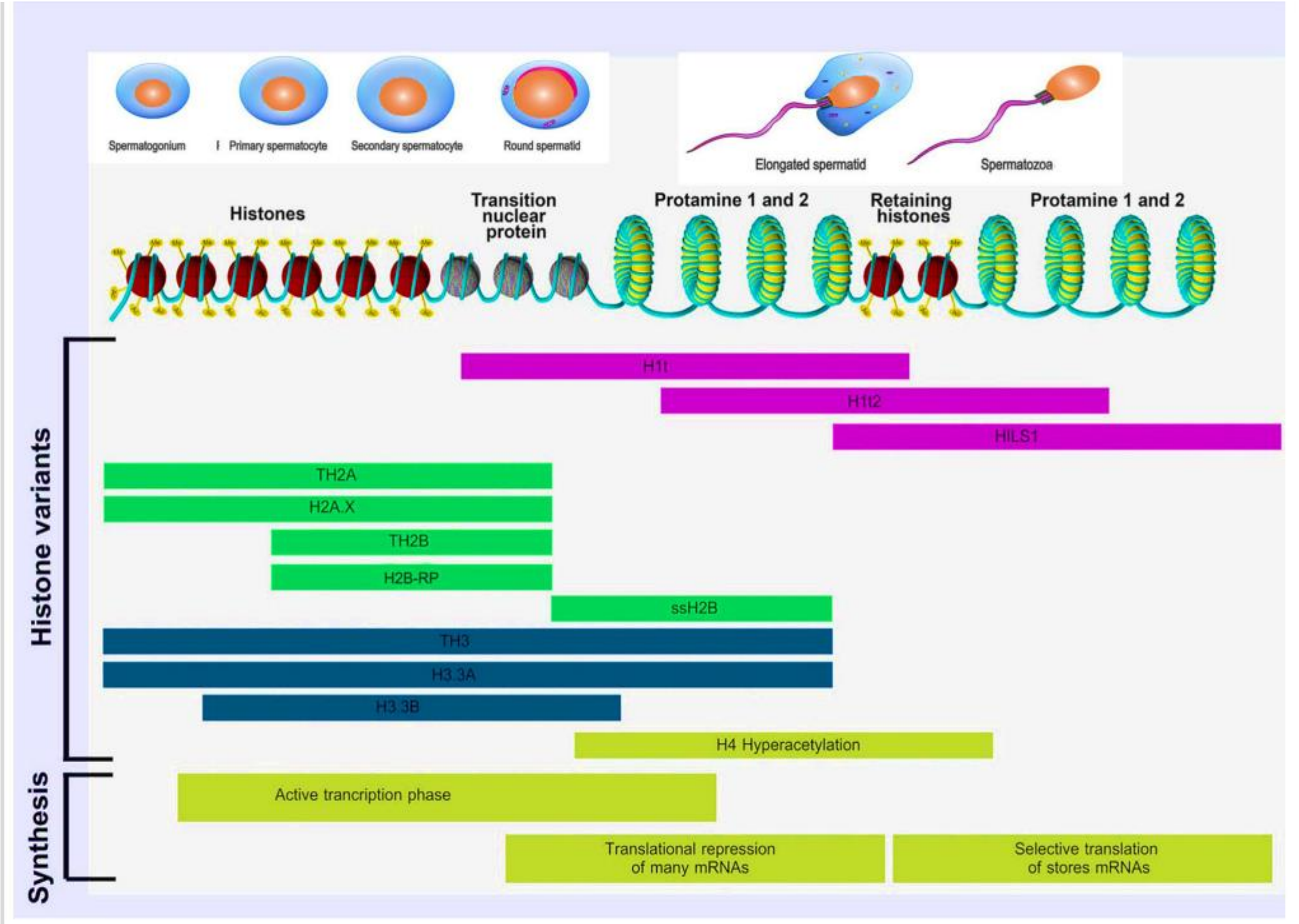

Figure 2 - Histone changes from spermatogonia to spermatozoa (adapted from Kimmins and Sassone-Corsi, 2005; Rathke et al., 2005; Özbek et al., 2021).

Sperm proteins

Proteins from sperm and their relationship with fertility are presented in Table 1. The ability of sperm to fertilize an egg is determined by the role of sperm proteins. Proteins such as ODF2, PAWP, PRM, TH2B, PEBP4, and UQCRC2 are sperm proteins associated with fertility. Sperm contains intracellular proteins, membrane proteins from the cell, and proteins bound to them from fluid derived from the epididymal and auxiliary sex glands (Rego et al., 2016; Kenny and Byrne, 2018; van Tilburg et al., 2021).

Outer Dense Fiber protein 2 (ODF2) is the protein detected in sperm and is associated with fertility. Kaya et al. (2021) reported that ODF is found in the sperm tail axoneme. In sperm, ODF2 is a cytoskeletal structural protein in flagella. The ODF2 signal is strongest on the main tail, followed by a decrease in strength at the tip and no signal in the middle, indicating that the mitochondrial sheath prevents anti-ODF antibodies from reaching the ODF fibers. The ODF2 gene produces a protein that is a major component of the sperm tail (Hoyer-Fender et al., 1998; Salmon et al., 2006; Hüber et al., 2008). ODF2 consists of nine fibers with doublet tubules of axonemy on the outer edge (Donkor et al., 2004). ODF2 has a function in maintaining the sperm tail which is needed to protect the sperm tail from shearing forces during the epididymis and ejaculation process (Kumar and Singh., 2021).

Protamines (PRMs), the key proteins in spermiogenesis for chromatin condensation, are potential candidate genes for sperm motility markers (Kumar et al., 2018) and as a protein biomarker of semen quality and production (Pardede et al., 2020). In the sperm head, PRM is one of the most prevalent core proteins. In humans (Balhorn et al., 2018), mice, rats, and hamsters (Bower et al., 1987), two kinds of PRM, namely PRM1 and PRM2, have a role in sperm function. According to Beletti et al. (2005), only one type of PRM, PRM1, plays a dominant role in the normal operation of bull sperm. However, PRM1, PRM2, and PRM3 are all expressed in bovines, according to Ferraz et al. (2013). Protamine is an arginine-rich proteins required for efficient compression of sperm DNA into a 10-fold more compact condition, compared to other spermatogenic cell types such as spermatogonia, until protamine toroid activities cause differentiation into spherical spermatids (Ward, 2010). During spermiogenesis, PRM replaces histone somatic cells in a complicated process (Bao and Bedford, 2016).

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Testis specific histone 2B (TH2B) is a biomolecular marker that can be used to assess the quality of sperm and predict bull fertility and sire suitability for artificial insemination (AI). Kutchy et al. (2017) reported that TH2B is localized in the sperm head and is related to sperm chromatin dynamics and bull fertility. Shinagawa et al. (2015) found that TH2B creates an open structure in sperm chromatin and engages in the replacement of proteins between the nuclei. During the process of spermatogenesis, chromatin undergoes structural reorganization. TH2B is involved in remodeling chromatin structure during spermatogenesis (Lu et al., 2009). Histone variant from TH2B is identified as testis H2B (Trostle-Weige et al., 1982). TH2B affects sperm chromatin's inter-nuclear protein replacement and produces open chromatin shape (Shinagawa et al., 2015).

Phosphatidylethanolamine binding protein 4 (PEBP4) has been identified as a secretory protein with an N-terminal signal peptide involved in serine protease control (Wang et al., 2004). PEBP is a highly conserved protein in mammals that is mostly expressed in the testis, as well as the parathyroid gland, spleen, gallbladder, small intestine, salivary gland, rectum, stomach, and kidney (Uhlen et al., 2015). PEBP4 enhanced sperm motility in boar sperm, hence it's possible that PEBP4 regulates sperm production and maturation in bovines (An et al., 2012). PEBP4 protein was higher in the high-fertility bulls group compared to the low-fertility and infertile groups (Somashekar et al., 2017; Selvaraju et al., 2018). PEBP4 in the seminiferous tubules' elongated spermatids and Leydig cells but not in the Sertoli or spermatogonia cells. PEBP4 also affects sperm metabolism and motility via protein phosphorylation signaling (Silva et al., 2015). In addition, PEBP4 inhibits serine proteases and protects cells against TNF-induced apoptosis (Wang et al., 2014), and it is linked to spermatogenesis and sperm motility (Somashekar et al., 2017). PEBP4 has also been annotated particularly in relation to fertility traits like age at puberty and spermatogenesis (Somashekar et al., 2017; Stafuzza et al., 2020).

Ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRC2) has been reported to be correlated with bull fertility (Park et al., 2019). Park et al. (2019) revealed that UQCRC2 can be used to predict below-normal bull fertility. After capacitation, UQCRC2 was shown to be strongly expressed in sperm with large litter sizes (Kwon et al., 2015). UQCRC2 is a protein involved in the electron transport chain's (ETC) complex III and IV assembly (Lopes et al., 2021), associated with oxidative stress (Shibanuma et al., 2011) and oxidative phosphorylation (Filipović et al., 2020). Increased ROS generation occurs due to UQCRC2 deficiency (Park et al., 2012). UQCRC2 was reported to be significantly lower in high-fertility bulls than in low-fertility bulls (Park et al., 2012).

Table 2– Sperm proteins

Preferred name	Annotation	Function	References
ODF2	Outer Dense Fiber protein 2	Activation and fertilization	Kaya et al., 2021
PRM	Protamine	Changes in histones in sperm spermatid chromatin to the developmental stage of spermatogenesis, to condense sperm DNA into a complex, dense, and stable condition	Fortes et al., 2014; Dogan et al., 2015
TH2B	Testis specific histone 2B	Testicular-specific histone variants required for the transformation of dissociated nucleosomes to protamine in male germ cells	Kutchy et al., 2017
PEBP4	Phosphatidylethanolamine binding protein	Regulation of sperm motility and fertility	Somashekar et al., 2017
UQCRC2	Ubiquinol-cytochrome-c reductase complex core protein 2	Component of the multisubunit transmembrane complex known as the ubiquinol-cytochrome c oxidoreductase, which powers oxidative phosphorylation as part of the mitochondrial electron transport chain	Park et al., 2012

Table 3– Seminal plasma proteins

Preferred Name	Annotation	Function	References
OPN	Osteopontin	Sperm viability	Erikson et al., 2007
PLA2	Phospholipasea 2	Acrosome reaction	Kumar et al., 2012
P25b	P25b	Fertilizing ability	Kumar et al., 2012
aSFP	Acidic seminal fluid proteins	Motility and freezability	Kumar et al., 2012
a-L-fucosidase	Alpha-L-fucosidase	Fertilizing ability	Kumar et al., 2012
BSP	Bovine seminal plasma	Maturation process	Manjunath et al., 2009

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Seminal plasma proteins

Proteins from seminal plasma and their relationship with fertility are presented in Table 2. Proteins such as OPN, PLA2, P25b, aSFP, a-L-fucosidase, and BSPs are seminal plasma proteins associated with fertility.

The candidate specification associated with fertility is osteopontin (OPN). OPN is an extracellular phosphoprotein matrix protein that releases identified chemicals in various tissues and fluids, including those of the male and female reproductive tracts. OPN was previously identified as a marker of high fertility with a molecular weight of 55 kDa in Holstein bull seminal plasma, produced by the ampulla and vesicular glands (Erikson et al., 2007). In a previous study of OPN, a 55 kDa isoform was detected in seminal plasma in Holstein bulls, which had a positive correlation with fertility (Cancel et al., 1997). OPN can be found in bull's accessory sex glandular fluids (AGF), seminal vesicle fluids and ampullary fluids (Cancel et al., 1997), the epithelium of male reproductive tract in humans, and rat's testis (Brown et al., 1992) and epididymis (Manjunath, 1984; Siiteri et al., 1995; Luedtke et al., 2002). However, previous studies were unsuccessful in detecting OPN in immunofluorescent bovine sperm. According to Cancel et al. (1997), OPN has several relationships and functions in ejaculated bovine sperm due to its presence in the male reproductive tract and seminal plasma, as well as its correlation with male fertility. OPN has been described as a sperm surface molecule in mice (Siiteri et al., 1995), such as those associated with sperm during development in the testes (Luedtke et al., 2002) and while sperm are transported and stored in the epididymis (Manjunath, 1984; Luedtke et al., 2002) and are present in AGF (Cancel et al., 1997). Therefore, the AGF protein is known to bind to sperm during ejaculation (Weinman et al., 1986). Osteopontin was also reported to be positively correlated with freezability of bull (Rego et al., 2016), several peptides (Willforss et al., 2021), sperm-egg binding and embryo development (Moura, 2005; Erikson et al., 2007; Gonçalve et al*.,* 2008; Monaco et al*.,* 2009).

Phospholipase A2 (PLA2) is found in the plasma membrane, acrosome, and post-acrosome substantion of ejaculated bull sperm (Weinman et al., 1986). The molecular weight of 60 kDa and pI 5.6 (Soubeyrand et al., 1997) and the 16 kDa PLA2 isoform were also detected (Ronkko et al., 1991). It was demonstrated that PLA2 adhered to the surfaces of ejaculated bull sperm but not epididymal sperm (Ronkko, 1992). PLA2 forms a superfamily of proteins, hydrolyzes the sn2-ester bonds of glycerophospholipids, and is involved in many biological functions (Six and Dennis, 2000). PLA2 activity in seminal plasma and sperm heads is known to be associated with motility and fertility (Anfuso *et al.,* 2015). PLA2 is also involved in capacitation, acrosome reaction, and early stages of fertilization, sperm binding and sperm-oocyte fusion in mammals (Roldan and Fragio, 1993; Pietrobon et al., 2005; Roldan and Shi, 2007; Stival, 2016). Furthermore, PLA2 is classified into PLA2 (cPLA2), PLA2 (sPLA2), PLA2 (iPLA2), and platelet-activating factor (PAF) (Alberghina, 2010). PLA2 is a protein found mostly in male reproductive organs (Koizumi et al., 2003; Bao et al., 2004; Masuda et al*.,* 2005; Roldan and Shi, 2007).

P25b is a sperm protein found in bulls that is associated with the acrosome's plasma membrane. P25b is part of the xylulose reductase family, which is secreted by the epididymal epithelium and adheres to the sperm surfaces of the testes during epididymal transit. This corresponds to the plasma membrane that covers the acrosomal caps of the sperm (Khumran et al., 2020). Through apocrine secretion by epithelial cells bordering the epididymal lumen, epididymosomes are released in cauda epididymal fluids. Epididymosomes transfer these proteins from the epididymal lumen to the sperm surfaces depending on pH, temperature, and zinc (Frenette et al*.,* 2002). Karunakaran and Devanathan (2017) reported that P25b was only present in high-fertility bulls and absent in low-fertility bulls. P25b plays a key role in the regulation of cellular activities that lead to acrosome reaction, recognition, binding, and sperm penetration of the oocyte's zona pellucida during fertilization (Iida et al., 1999; Girouard et al., 2009). Kumar et al. (2012) identified P25b as a potential sperm maturation and fertility marker associated with freezing and thawing techniques.

Acidic seminal fluid proteins (aSFP) are a protein family that significantly affects in vitro mitogenicity and steroidogenesis (Einspanier et al., 1993). aSFP, also known as Spermadhesin-1, is secreted by the accessory sex glands (Moura et al., 2007) and the cauda epididymis of bulls (Moura et al., 2010). aSFP has the ability to guard against oxidative stress triggers in semen; the effects of aSFP on sperm motility and mitochondrial activation are very important, when aSFP is at high concentrations (Einspanier et al., 1993; Schoneck et al., 1996).

Alpha-L-fucosidase was found in seminal plasma, epididymal fluid, and bull sperm (Jauhiainen and Vanha-Perttula, 1986; Srivastava et al., 1986). Cauda epididymidis is the main source of α-L-fucosidase in bulls (Srivastava et al., 1986). Moura et al. (2006) also detected α-L-fucosidase in the cauda epididymal fluid of bulls which had a molecular weight of 54.4 kDa and pI of 6.6 and was associated with high bull fertility. α-L-fucosidase involved in the modification of the carbohydrate portion of the sperm membrane protein during epididymal transit and is found in lower numbers in the seminal plasma of bulls with a higher percentage of abnormal sperm (Jauhiainen and Vanha-Perttula, 1986). Pure enzymes promote acrosome reactions of guinea pig sperm in vitro (Srivastava et al., 1986).

Bovine seminal plasma (BSP) is one of the proteins of the seminal plasma associated with fertility. BSP proteins are a subfamily of proteins that play a key role in sperm maturation and have been extensively researched in terms of biochemical, structural, and functional characteristics (Manjunath et al., 2009). They also take a pivotal part in the formation of the oviductal sperm reservoir (Gwathmey et al., 2006). BSP secreted by the seminal vesicle belongs to the heparin-binding protein family and represents approximately 70% of total protein content of bovine seminal plasma (Nauc and Manjunath, 2000). Bovine seminal plasma contains a protein family designated as Binder of Sperm (BSP) protein,

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which has been characterized extensively (Calvete et al., 1995; Bourgeon et al., 2004). The BSP family of proteins is found in mammalian seminal plasma in various forms and is ubiquitous in nature (Villemure et al., 2003). The BSP protein superfamily includes the BSP-1 (PDC-109), BSP-3 (BSP-A3), and BSP-5 (30 kDa) proteins, which are found in all mammals and are involved in various fertility-related events such as sperm membrane modification during capacitation and acrosome reaction, and sperm motility maintenance during oviduct storage by binding to the oviductal epithelium (Nauc and Manjunanth, 2000; Gwathmey et al., 2006; Souza et al., 2008).

CONCLUSION

Research related to protein, specifically in bulls, would be very helpful to better understand and confirm fertile, infertile or subfertile bulls with various conditions in the field. However, proteins have been reported in sperm and seminal plasma that can assist in the selection of fertile bulls.

DECLARATIONS

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Authors' contribution
All authors contributed equally in conducting and writing the manuscript.

Conflict of interests
The authors declared that they had no conflicts of interests.

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CONNECTION BETWEEN GENE MARKERS WITH MILK PRODUCTION TRAITS OF UKRAINIAN DAIRY COWS

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ABSTRACT: The purpose of this study was to obtain information on the genotyping of cows of Ukrainian dairy breeds for the presented loci capa-casein gene (CSN3), thyroglobulin (TG-5) gene, leptin (LEP), pituitary-specific transcription factor (Pit-1), and beta-lactoglobulin (BLG) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and to test their association with milk production. The influence of the genotype on milk yield, fat and protein content in the milk of cows of Ukrainian selection has been established. The highest levels of milk productivity traits were expected from animals with the CT genotype for the LEP gene, AA for the CSN3 gene, CC for the TG5 gene, and AA and BB for the Pit-1 gene. But at the same time, milk obtained from cows with CSN3 BB genotype were characterized by the best cheese suitability. The effect of the homo- or heterozygous state of BLG on the signs of milk production was less noticeable, and the homozygous TT genotype for the TG5 gene was not found in any of the studied breeds. The results obtained can be used in the practical work of breeding farms along with traditional methods of selection, control and preservation of the genetic diversity of specific herds at an optimal level.

Keywords: Capa-casein, Marker genes, Polymorphism, Thyroglobulin, Leptin, Pituitary transcription Factor, Beta-lactoglobulin

INTRODUCTION

In recent decades, advances in DNA-based marker technology have been able to identify regions of the genome (namely, quantitative trait loci, QTLs) underlying complex traits such as milk yield. Instead of traditional animal breeding programs based solely on phenotype and pedigree information, the application of identified QTLs to genetic evaluation provides great potential to improve selection accuracy, thereby could accelerate genetic improvement in animal productivity (Jiang et al., 2010; Kumar, 2017; Kramarenko et al., 2019).

The introduction of the molecular genetic methods in animal husbandry is associated with the development of PCR technology, made it possible to conduct a quick analysis of the connection between allelic variants of genes with productivity. With the development of DNA-based marker technology, it has become possible to identify regions of the genome (i.e., loci of quantitative traits, QTLs) that are associated with complex traits. The inclusion of the detected QTLs in the genetic evaluation allows a clear increase in selection accuracy, thereby accelerating the genetic improvement of the productive qualities of the animals. Numerous studies have been published in recent decades to identify QTLs for signs of dairy livestock dairy productivity. Advances in the detection of causal genes for complex such are slow, as association mapping results in large confidence intervals. In particular, the region in which QTL is mapped may contain a large number of possible candidate genes (Wang et al., 2014; Kusza et al., 2015). Genome-wide association studies (GWAS), based on high-throughput single nucleotide polymorphisms (SNPs) genotyping technologies, open up ample opportunities to study genes associated with signs of livestock dairy productivity, as confirmed by a number of domestic and foreign scientific studies (Arora and Bhatia, 2004; Soltani-Ghombavani et al., 2013; Zhou et al., 2019).

Breeding farm animals is an integral part of a complex breeding system. When evaluating animals of dairy breeds, not only a high level of milk productivity, but also quality indicators of milk are of great importance. Increasing yield, fat content in milk, live weight of cows is considered the classic direction of work of genetic scientists. But at the same time, there are few works, especially domestic ones, devoted to the study of marker genes associated with the protein content in milk. Although the protein content and amount in milk, its structure is of great economic importance to the processing industry. Since the consumption of raw materials, time and energy resources for the production of dairy products depend on the fat content in milk, in addition, the protein content indicator largely determines the quality of finished products (Yurnalis et al., 2013; Lu et al., 2021).

The association of polymorphism in the 3 'region of the oxidized low density lipoprotein receptor 1 (OLR1) gene with milk fat and protein in active Holstein cows of Irish selection was studied by Soltani-Ghombavani et al. (2013). Thus, scientists have found that the OLR1 is the main receptor on the cell surface for the oxidized low-density lipoprotein, actively participates in the metabolism of lipoproteins and affects the protein content in milk. According to the authors,

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the OLR1 gene may be a marker gene that is associated with the production of protein content in milk. Because the genetic linkage phase can be different across breeds and populations, the use of previously identified markers to conduct marker selection is problematic, especially when marker density was low during discoveries. Consequently, high-density SNP GWASs are essential for understanding the genetic architecture of important and complex traits in cattle breeds.

A genome-wide association of milk research was carried out by Chinese scientists in Xinjiang Brown cattle of a combined direction of productivity. In their studies, the scientists evaluated five milk productivity measures: milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP) and protein percentage (PP) in Chinese cattle breed Xinjiang Brown. The authors found two very significant SNPs associated with milk composition characteristics. One SNP is associated with a percentage of fat and is located in the cadherin-2 (CDH2) gene at 29.1 Mbp on BTA 24. The cadherin-2 is a protein coding gene and is involved in adipogenesis. Depressing the CDH2 to block the epithelial-mesenchymal reaction can weaken the production of fat content in milk. Another milk-related SNP that the authors identified was significantly associated with protein content in milk and mapped at 75.8 Mbp on Bos taurus (BTA 7), which is in a gene called the Gamma2 receptor gamma-aminobutyric acid type A subunit (GABRG2). GABRG2 primarily promotes the activity of the gamma-aminobutyric acid-controlled chloride ion channel (GABA) and is involved in the activity of the GABA-A receptor and promotes the production of the amount of protein in milk (Zhou et al., 2019; Zhang et al., 2022). Scientific research on the assessment of annual genetic progress and economic efficiency becomes the basis for the development of methods for optimizing long-term breeding programs for dairy cattle, while improving methodological approaches to solving this problem. Wang et al. (2014) established a significant association between SNPs in the histidine ammonia-lyse gene (HAL) gene and signs of cow dairy productivity of the Chinese Holstein breed, indicating the potential role of HAL variants in these signs. These identified SNPs may be genetic markers used in genomic selection schemes to accelerate the genetic growth of dairy livestock productivity traits.

Thus, domestic and foreign experience shows that the effectiveness of selection depends on many genetic, paratypical and economic factors (Ng-Kwai-Hang et al., 1984; Lund et al., 2011; Kramarenko et al., 2019; Lu et al., 2021; Pedrosa et al., 2021). The possibility of purposefully creating a highly productive animal population is significantly correlated with the presence of information about genes that control signs of productivity. In this regard, the task of identifying and using marker genes responsible for the manifestation of economically valuable signs becomes urgent.

The composition of milk is an important breeding characteristic of dairy breeds of cattle, especially in the conditions of modern animal husbandry; therefore, the task arises to analyze the influence of polymorphism of protein and lipid metabolism genes on the formation of milk productivity indicators in cows of the dairy direction of domestic breeding productivity.

MATERIALS AND METHODS

Materials

To conduct the study, experimental groups were formed from breeding cattle of the dairy direction of productivity - Ukrainian Red Dairy (URD, n = 32 heads), Ukrainian Black-speckled Dairy (UBSD, n = 32 heads), Ukrainian Red-speckled Dairy (URSD, n = 28 heads) breeds of the leading enterprise in the south of Ukraine PSP “Kolos-2011” Ochakov region.

Ethical regulation

The rules for handling animals in experiments comply with European legislation on the protection and comfort of animals kept on farms (Directive No. 95/58 EU "On the Protection of Farm Animals" of the Council of the EU of 20.07.1998, as amended by EU Regulation No. 806 / 203 of 14.04.2003, No. 91/630 EU "Minimum standards for the protection of pigs" of 19.11.1991, as amended by the EU Regulation. Pilot study protocol for blood sampling in cows approved by the local commission on bioethics of the National University of Life and Environmental Sciences Ukraine on Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals.

Methods

The characterization of the polymorphism of the genes studied was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Grodzicker et al, 1974; Alexander et al., 1988; Pedrosa et al., 2021). Genomic DNA was isolated from the peripheral blood of animals according to the method of Kostyunina et al. (2020) and using the standard commercial kit "DNA Sorb B" manufactured by Amplissens (Russia), according to the manufacturer's recommendations (Pedrosa et al., 2021). The DNA concentration was tested by electrophoresis in a 2% agarose gel. To carry out the polymerase chain reaction, a reaction mixture of 10 µL was used in operation: dH2O - 4.3 µL, PCR buffer 5 (15 mMg-1.0 mL) - 2.0 µL; Deoxynucleotide triphosphates (dNTP) mixture of 10 (2 mM each) - 0.8 µL; two primers (70 ng each) - 0.8 µl; Taq polymerase (1 ml/1000 U) - 0.1 µL; DNA 50-100 ng - 2.0 µl.

Restriction products were separated by electrophoresis in 2% agarose gel followed by staining in ethidium bromide solution. Visualization was carried out on a transciluminator in UV light, followed by electropherogram photography by a digital camera. The size differentiation of amplicons was carried out using the molecular weight marker Gene Ruler TM 50 bp DNA Ladder, SM0378 (Fermentas®, Lithuania; Oztabak et al., 2008). The temperature regime and the number of PCR amplification cycles for each gene were determined separately. For the analysis of the polymorphism of the structural loci k-Cn, βLG, TG, Pit-1, and LEP, restriction enzymes matched for each locus were used; typed immediately after PCR analysis (Grobet et al., 1998).

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Statistical analysis
Statistical data processing was carried out in the standard package Microsoft Excel-2013.

RESULTS AND DISCUSSION

Identification of selection signatures allows a better understanding of the evolutionary processes, functions and organization of genes in the genome. Of particular note are genes, certain allelic variants of which are associated with the quality of raw materials. In cattle, genes whose polymorphism is associated with dairy productivity are known and investigated by us (Figure 1).

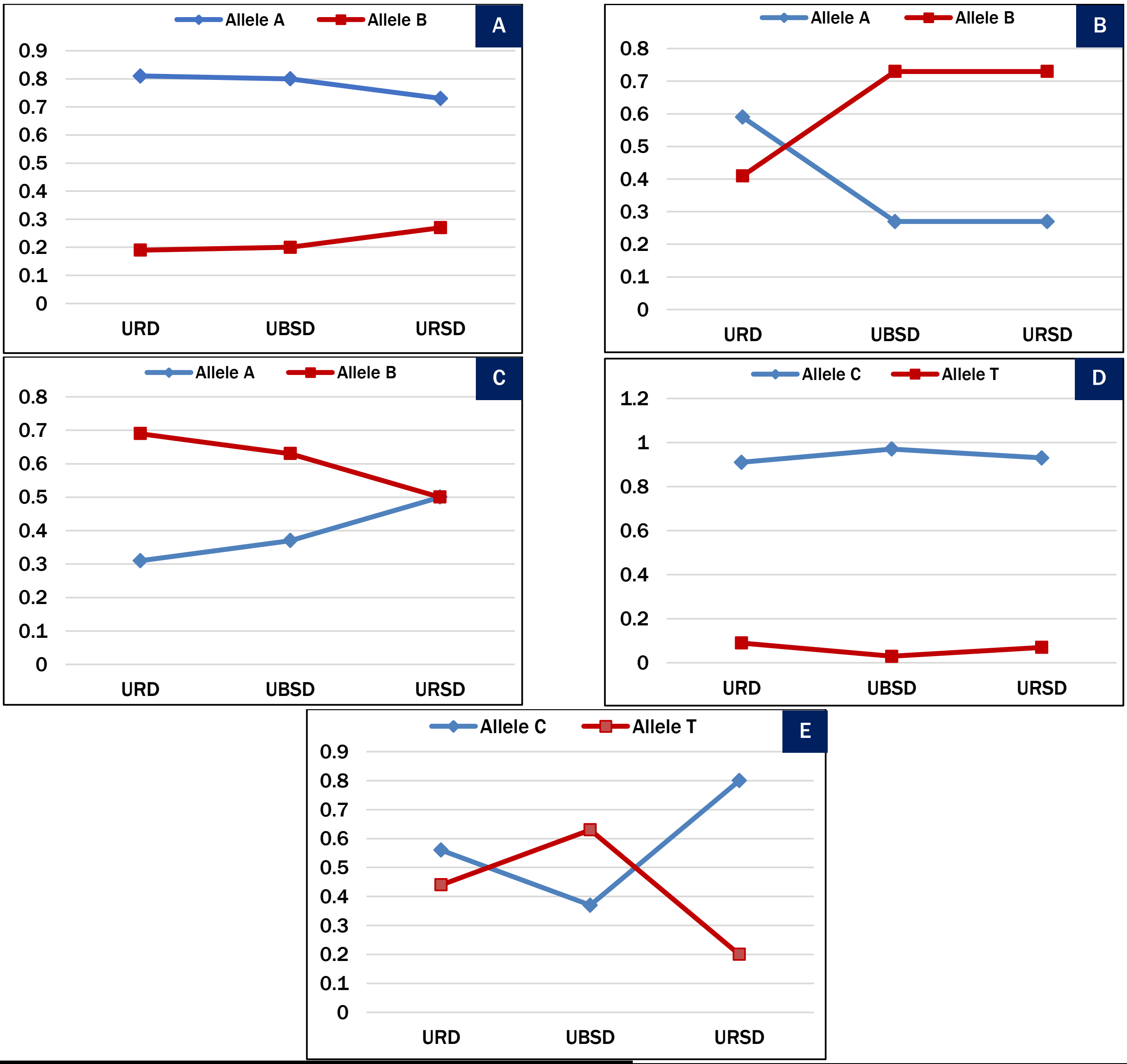

Figure 1 - Distribution of allelic variants frequencies by loci in cows. A: Capa-casein gene (CSN3); B: Beta-lactoglobulin (BLG); C: Leptin (LEP); D: pituitary-specific transcription factor (Pit-1); E: TG5 thyroglobulin (TG-5) gene. Ukrainian Red Dairy (URD): n=16, Ukrainian Black-speckled Dairy (UBSD): n=15; Ukrainian Red-speckled Dairy (URSD): n=15.

The studied genes are divided into several groups: A) milk protein genes, which affect the protein content in milk, its technological properties, quality and yield of protein-containing products; B) lipid metabolism genes synthesized in adipocytes, are responsible for regulating the body weight of the animal, its consumption of feed and its fat deposits, and are also involved in the synthesis of milk fats and genes of regulatory systems, which generally affect the productive performance of the body and serve as a somatic regulator of animal growth (Khatib et al., 2007; Miluchová et al., 2018).

That's how, capa-casein (CSN3) is connected with milk protein and its coagulation characteristics, including the role of a stabilizing factor in the formation of micelles, blocking their aggregation. And when it is dissolved, milk coagulates, casein precipitate formation and clot formation and affects the mass share of fat in milk, the volume of milk yield. Beta-lactoglobulin (BLG) is associated with a higher concentration of fat and protein in milk and has a significant effect on the creation of active immunity in calves. Meanwhile, as a pituitary-specific transcription factor (Pit-1) is known for the function of increasing milk yield and fat yield. Leptin provides formation of fat deposits and increases productivity in

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terms of fat content in milk during the first lactation period of livestock. Thyroglobulin (TG) affects the total percentage of fat in tissues, including milk formation (Hartati et al., 2018).

The results of analysis of allelic variants distribution of the investigated structural genes of protein and lipid metabolism in the above rocks are presented in the Tables 1 - 5. Studies of associations between productivity indicators and different genotypes of loci made it possible to establish a difference in the dependence of individual genotypes and signs of livestock breeding - yield, the content of fat and protein in milk depending on the breed belonging of cows and their individual characteristics.

Analyzing the productivity indices at the locus of the leptin gene, we found that cows of three breeds that were included in the study with the CC genotype had a significantly higher ($P < 0.05$) fat content in milk than cows with the CT and TT genotype (behind the higher lactation index, Table 1). At the same time, no significant difference was found between the control and experimental groups in the test animals of the above-mentioned genotypes in terms of fat content and their age no significant difference was found between the control and experimental groups.

The results of evaluation of experimental animals by the locus of the leptin gene by the parameters of utilization depending on the genotype indicated that the best were representatives with the available alleles of the breed URSD CT and exceeded the URD taken as a control group: for the first lactation by 1425 kg of milk, for the second by 3006 kg, above 825 kg ($P < 0.05$). In terms of protein content in milk, all animals are inferior to the control group in the range from -0.01 to -0.28% ($P < 0.001$) (Table 1), however, this trend is not traced in terms of protein milk content due to their milk yield. Thus, the indicators of all groups of cows at the locus of the leptin gene that were included in the study, for the first, second third and higher lactation, tend to increase their productivity, which is explained by their physiological maturity and the development of productive qualities with age. And in the context of experimental groups, heterozygous genotypes of CT turned out to be better.

Studies of the association of the leptin gene with productivity and reproducibility were carried out by Liefers et al. (2005). Thus, the authors found that the polymorphism located on intron 2 of the leptin gene explains a significant part of the variability in milk yield. An SNP associated with the first postpartum luteal activity (FPLA) was found in the propromotor region of the leptin gene. This SNP could be a potential marker of dairy cow fertility. Another SNP on the leptin promoter was associated with energy balance and dry matter intake (DMI), where high dry matter intake occurred together with higher energy balance. The scientists identified two genotype combinations of the aforementioned three associated leptin SNPs that had high hopes along with good energy balance and fecundity. Kononoff et al. (2005), observed an association between leptin genotype and carcass characteristics in beef cattle. The carcass mass of animals with CC genotype tended to increase than that of animals with TT genotype (365.5 versus 362.3 kg). No significant difference in carcass weight between TT and CT genotypes was observed.

In the course of cow productivity analysis of all three test breeds of Ukrainian selection by capa-casein locus Table 2, it was established, that individuals with AA genotype had higher yield rates, compared to the peers who were inherent in the AB genotype from 250 kg to 784 kg considering, that the yield level of the control group of the breed URD for higher lactation was 7943 ± 793.1 kg ($P < 0.05$). The highest level of hoot in the section of the studied breeds was characterized by cows of the Ukrainian Black-speckled Dairy breed, which had allele A in their genotype, compared to individuals who had allele B in the genome. It should be noted that only representatives of the Ukrainian Red-speckled Dairy breed are characterized by the presence of females with a homozygous BB genotype can be explained by their origin.

The revealed trends of intergenerational differentiation by fat and protein content in milk of cows with different genotypes by the capa-casein locus are multidirectional and statistically unreliable. In our opinion, this is due to the small number of groups of studied animals that are carriers of this genotype, the frequency of which is low in the populations of the breeds that were studied. Thus, we have established a relationship between economically useful signs of cow genotype by gene kapa-casein. Namely, the animal homozygous genotype AA was characterized by the inherent manifestations of milk productivity according to the signs of hoax, fat and protein content, as well as their amount in milk, compared to other genotypes, both in the context of experimental breeds and in their age dynamics. But the best raw suitability was characterized by milk obtained from cows with the BB genotype. It is also confirmed by Morkūnienė et al. (2016) which investigated the polymorphism of the kappa-casein gene of Lithuanian dairy cattle. They found that the most common genotype of AA, which had 49.2% of the studied animals. The greatest influence on the processing properties of milk was the BB genotype, found only in 2.1% of cows (Morkūnienė et al., 2016). Similar data were obtained by Machulnyi (2018), in herds of red steppe, Ukrainian Black-speckled Dairy and Ukrainian Red Dairy breeds of different body formation intensities. Representatives of homozygous AA genotypes were characterized by higher milk productivity, and the number of cows with this genotype is significantly higher in the herd, than the proportion of the capa-casein gene more technologically valuable B allele, but at the same time milk of URD and UBSD of cattle of slow formation intensity, and a rapid growth type URD will provide the highest yield of the final product within 10%, although the dairy productivity of such animals has not always been high.

When evaluating the cows of the experimental groups by the locus of the thyroglobulin gene, there was a tendency to increase the level of impact with the CC genotype, while the animals of the breed UBSD probably exceeded the control group in terms of impact (for the first lactation) by 760 kg ($P<0.05$). At the same time, in terms of protein content in milk, no significant difference was observed in cows with different genotypes for the thyroglobulin gene. It should be noted that in all three groups of cows there were no individuals with the TT genotype (Table 3). This indicates a low frequency of this genotype in the studied animal populations and is presumably associated with their origin.

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In none of the studied populations of cows of Ukrainian selection, there was a significant difference between the fat content in milk among carriers of different genotypes according to the thyroglobulin gene. Although in UBSD cows carriers of the homozygous CC genotype for the TG5 gene, there was a tendency to increase milk fat content with an insignificant difference relative to the control data. But at the same time, the study and analysis of the genetic structure for the polymorphism of the TG5 gene for the leading dairy breeds of Ukraine confirmed the positive effect of the C allele on the increase in fat content in milk. Therefore, the increase in its frequency in populations of Ukrainian dairy breeds is probably due to the influence of various artificial selection factors based on the signs of inductance, the manifestation of which is associated with the polymorphism of this gene. Thus, the effect of homo- or heterozygosity of the thyroglobulin gene on most productive features of cows was established, both in the cross-section of breeds and in age dynamics. Cows that were carriers of the homozygous CC genotype are characterized by higher values of milk productivity in the section of the first, second, third and highest lactation, regardless of the breed affiliation of the cows. At the same time, another homozygous genotype of TT was not found in any of the studied breeds of UBSD, URSD and URD, which, in our opinion, is associated with the individual characteristics of these breeds and their origin, and high homozygosity according to the allele C.

There are also contradictory data of Khatib et al. (2007), who did not reveal the relationship between milk yield and milk composition with Holstein dairy cattle and the TG5 gene. A, Putra et al. (2019) found in Indonesian cattle that the TG5 gene plays an important role in the regulation of metabolism and affects the differentiation of adipocytes, growth and homeostasis of fat depots and, as a result, contributes to an increase in milk productivity of cows in such an indicator as fat content in milk. Similar data were obtained by Dubey et al. (2015) on dairy breeds of cattle Mehsana and Nili Ravi.

A study of animals with different genotypes according to the β-lactoglobulin gene showed that there was no obvious change in the increase or decrease in the milk productivity level and fat content in milk depending on the genotype in the section of research breeds of cows (Table 4). But a tendency to an increase in the protein content in milk in cows of the UBSD breed was revealed by the AB genotype by the β-lactoglobulin locus, which is statistically not reliable. Thus, the effect of the homo- or heterozygous state of BLG globulin on signs of milk productivity is less noticeable. Thus, cows of UBSD (II lactation), URSD (III lactation) with genotype AA were characterized by somewhat reduced fatigue, and the largest representatives of the first group with genotype AA were alternately during ontogenesis, with the exception of II lactation. In general, homo and heterozygous genotypes of UBSD and URD of rocks of different combinations were observed with higher productivity values.

Mixed results were also obtained by Indian scientists (Bangar et al., 2021). The results of their studies also showed that the BLG gene variants had an inauthentic association with fatigue in all genetic models. Although positive effects of BLG in some models were observed, they did not correspond to statistical significance due to high heterogeneity between studies concluding that BLG genotypes had an uncertain effect on milk yield. It was concluded that BLG markers can also be useless for increasing milk yield in Indian dairy cows. Contrasting study results were obtained by Ozdemir et al. (2018), establishing a valid relationship between BLG genotypes and daily fatigue, fat and protein content in milk, and protein quantity. Which indicates that BLG-globulin genes are useful for improving the features of selection studied and can be used as molecular markers in predicting productivity. Cardona et al. (2016), also identified additive effects at the BLG locus for all signs of milk productivity during most lactation, but there was a dominant effect only in the early stages of fat release. For this gene, allele A had a favorable genetic effect throughout lactation on milk yield and quality indicators in tropical dairy goats.

Assessment of the productivity of cows of different genotypes according to the gene of the pituitary-specific Pit-1 factor showed that cows with BB genotype probably dominated peers with AA genotype in terms of hoax level (Table 5), this trend was observed in cows with UBSD their difference between the three research groups in terms of this indicator was in the range from 1000 to 1388 kg (P>0.95). The presence of allele B in heterozygotes AB predetermined the high milkiness of cows of experimental groups, all breeds included in the study without exception. And such animals exceeded the homozygous analogues with the AA genotype. By the mass fraction of fat in milk, a probable advantage of cows with an allele A in the genotype over peers was found for which the BB genotype was characteristic (P<0.05). According to the level of protein in the milk of cows belonging to three Ukrainian dairy breeds with different genotypes by gene Pit-1 we did not establish a reliable difference in favor of this or that group. At the same time, we did not find any homozygous genotypes of the AA variant in representatives of the red dairy breed by gene Pit-1. It seems that, there is association with the individual breed features of this cattle and gene mutation during the selection process at the stage of its creation (Li et al., 2021; Seo et al., 2022). Thus, the pituitary-specific factor Pit-1 gene was associated with high-yield animals with homozygous AA and BB genotypes for all lactation among livestock UBSD and URSD, while URD cows had the best BB genotypes, which also causes milk fat content in these animals. But the amount of fat did not differ significantly between homo- and heterozygous genotypes. Mattos et al. (2004) also established in the Gyr breeding bull population associations of Pit-1 with fat percentage and fat yield, respectively, in their daughters. Edriss et al. (2009) in studies of the association of PIT-1 gene polymorphism with birth weight, milk and reproductive qualities in cows of the Isfahan Holstein breed, the Pit-1 gene was considered as a marker candidate for milk production due to the regulation of expression of the bGH and prolactin gene necessary for breast and milk development. At the same time, negative data were obtained, as Aytekin and Boztepe (2013) noted that the polymorphism of the Pit-1 gene cannot be used as a candidate gene for the selection of dairy signs in brown Swedish cattle, since the authors did not find associations between the Pit-1 gene and signs of productivity.

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Table 1 - Association of LEP gene SNPs with signs of dairy productivity of cows

Characteristic, key figure	Locus genotype ($\bar{X} \pm S_x$) in the breed section								
	CC			CT			TT		
	UBSD	URSD	URD	UBSD	URSD	URD	UBSD	URSD	URD
(1 lactation)	2	10	4	6	4	10	7	1	2
Milk yield, kg	6646±908	5444±1055	6356±1706	6514±489	7220±1425*[p]	5348±960	6166±429	5299±0	5856±660
Fat content,%	3.81±0.110	3.77±0.106*[p]	3.78±0.165	3.87±0.027	3.48±0.321	3.81±0.117	3.84±0.071	3.60±0	3.79±0.040
Quantity of fat, kg	252.2±27.30	205.5±42.38	243.0±74.85	256.0±17.02	270.5±56	204.3±38.78	237.2±19.23	191 ±0	222.0±27.62
Protein content,%	3.20±0.008	3.10±0.070***	3.21±0.038	3.13±0.100	3.14±0.019	3.25±0.100	3.19±0.088	3.05±0	3.33±0.025***[a]
Amount of protein, kg	212.7±29.07	168.7±33.15	204.5±55.69	203.7±9.92	226.3±43.74	174.1±30.49	196.2±11.42	161.6±0	194.6±20.48
(2 lactation)	2	8	4	5	2	10	6	1	2
Milk yield, kg	5123±400	6179±1697	6897±1629	6988±895	8965±581	5959±903	7232±1193	6933±0	7759±1231
Fat content,%	3.77±0.020	3.74±0.098	3.69±0.080	3.77±0.137	3.63±0.030	3.77±0.112	3.81±0.132	3.80±0	3.62±0.015
Quantity of fat, kg	193.2±16.12	236.1±62.84	253.9±55.19	262.2±24.00	325.5±23.50	223.2±28.78	275.9±46.87	264±0	280.3±43.35
Protein content,%	3.20±0	3.10±0.075**	3.20±0.025	3.14±0.128	3.10±0.050*	3.23±0.105	3.18±0.083	3±0	3.30±0.035**[a]
Amount of protein, kg	163.9±12.82	191.3±52.74	220.6±52.27	218.5±23.08	277.6±13.53	192.6±31.42	228.5±32.38	208±0	255.2±21
(3 lactation)	2	6	2	3	0	8	5	1	2
Milk yield, kg	9327±1719	6475±617	5173±427	5920±1408	-	7768±1090	8049±1413	5438±0	8728±772
Fat content,%	3.84±0.075	3.79±0.153	3.86±0.085	3.61±0.029	-	3.78±0.203	3.73±0.122	3.87±0	3.69±0.190
Quantity of fat, kg	359±72.94	245.4±25.80	199.8±20.88	213.9±52.85	-	296.4±50.26	299.5±53.85	210.5±0	320.6±11.92
Protein content,%	3.10±0*	3.08±0.056*	3.21±0.055	3.17±0.111	-	3.23±0.081	3.16±0.088	3.0±0	3.18±0.075
Amount of protein, kg	289.1±53.30	199.8±22.02	165.5±10.86	188.4±51.10	-	250.8±35.87	253.0±39.22	163.1±0	276.5±17.98
(lactation veins)	2	10	4	6	4	10	7	1	2
Milk yield, kg	9327±1719	7856±1186	8036±978	7847±787	8046±1539*	7221±942	7860±1474	5438±0	9245±254.5
Fat content,%	3.84±0.075	3.69±0.066	3.84±0.103*[b]	3.71±0.087	3.62±0.073	3.69±0.102	3.64±0.069	3.87±0	3.62±0.020
Quantity of fat, kg	359.0±72.94	289.8±46.05	307.4±31.86	290.7±22.54	292.9±59.49	265.6±33.96	285.8±51.78	210.5±0	334.7±11.06
Protein content,%	3.10±0	3.08±0.074***	3.24±0.062	3.09±0.092**[a]	3.11±0.038	3.23±0.070	3.16±0.069	3.00±0	3.21±0.055
Amount of protein, kg	289.1±53.30	244.4±36.79	260.2±22.00	242.2±21.67	249.9±46.70	233.2±30.31	247.8±43.12	163.1±0	296.2±3.07

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

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Table 2 - Association of CSN3 gene SNPs with signs of dairy productivity of cows

Characteristic, key figure	Locus genotype ($\bar{X} \pm S_x$) in the breed section								
	AA			AB			BB		
	UBSD	URSD	URD	UBSD	URSD	URD	UBSD	URSD	URD
(1 lactation)	8	8	10	6	6	6	0	1	0
Milk yield, kg	6598±56	5999±1612**	6103±1152	6027±358***	5894±949	4932±528	-	5259±0	-
Fat content,%	3.81±0.068	3.64±0.233*	3.81±0.111	3.91±0.028**[a]	3.70±0.128	3.79±0.143	-	3.93±0	-
Quantity of fat, kg	251.4±22.54	225.8±63.88	233.8±50.61	239.7±19.53	218.9±41.47	186.8±20.90	-	207±0	-
Protein content,%	3.12±0.086	3.15±0.025	3.23±0.093	3.23±0.056	3.07±0.056	3.29±0.068	-	3.0±0	-
Amount of protein, kg	205.7±13.86	188.7±49.90	197.5±39.46	194.8±12.34	181.1±32.63	162.2±19.88	-	157.8±0	-
(2 lactation)	6	5	10	6	5	6	-	1	-
Milk yield, kg	7166±1317	7038±1627	6699±1187	6402±656	6136±1680	5950±1013	-	8381±0	-
Fat content,%	3.81±0.142**	3.69±0.098	3.67±0.102	3.78±0.104	3.73±0.087	3.83±0.080	-	3.83±0	-
Quantity of fat, kg	272.1±48.67	258.4±53.95	245.1±40.25	241.4±24.37	238.2±64.60	226.2±32.49	-	321.0±0	-
Protein content,%	3.11±0.102	3.15±0.060	3.21±0.094	3.23±0.058	3.05±0.060	3.26±0.060	-	3.00±0	-
Amount of protein, kg	222.1±38.32	220.2±49.16	215.6±40.89	206.0±19.68	188.1±52.91	193.8±33.09	-	251.4±0	-
(3 lactation)	4	2	6	5	4	5	-	1	-
Milk yield, kg	8638±1537*	7165±258	7854±1353	6693±1335***[a]	5932±777***[a]	6663±975	-	6228±0	-
Fat content,%	3.73±0.182	3.95±0.005	3.89±0.147	3.70±0.062	3.77±0.145	3.70±0.166	-	3.68±0	-
Quantity of fat, kg	323.3±23.22	282.7±9.84	308.4±60.98	248.2±51.88	222.1±20.72	245.9±34.86	-	229.2±0	-
Protein content,%	3.10±0.080	3.15±0.050	3.22±0.073	3.20±0.040	3.05±0.050	3.24±0.072	-	3.00±0	-
Amount of protein, kg	267.9±43.57	225,8±11.73	252.8±46.15	213.8±39.95	180.9±23.84	215.8±30.66	-	186.8±0	-
(lactation veins)	8	8	10	6	6	6	-	1	-
Milk yield, kg	8420±1358*	8193±1456	7943±793	7495±817	7650±825	7234±1017	-	6228±0	-
Fat content,%	3.68±0.099	3.68±0.094	3.75±0.116	3.71±0.068	3.68±0.058	3.66±0.122	-	3.68±0	-
Quantity of fat, kg	310.5±48.57	302.0±55.48	296.6±27.15*	278.0±30.59	281.3±30.89	264.8±36.11	-	229.2±0	-
Protein content,%	3.09±0.079	3.09±0.058	3.23±0.075	3.20±0.067	3.09±0.072	3.24±0.052	-	3.0±0	-
Amount of protein, kg	260.1±43.13	252.7±41.78	256.1±26.94	239.4±24.84	239.8±30.49	234.1±34.35	-	186.8±0	-

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to animals of the third control group); a= P<0.05; b= P<0.01. (in comparison of animals of the first experimental group with analogues of the second experimental group) Mean values with different superscripts in the column differ significantly (p<0.05). Capa-casein gene (CSN3); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD).

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Table 3 - Association of TG5 gene SNPs with signs of dairy productivity of cows

Characteristic, key figure	Locus genotype ($\bar{X} \pm S_x$) in the breed section								
	CC			CT			TT		
	UBSD	URSD	URD	UBSD	URSD	URD	UBSD	URSD	URD
(1 lactation)	14	13	13	1	2	12	0	0	0
Milk yield, kg	6436±540*	5985±1410	5676±1132	5433±0	5399±100.5	5607±1204	-	-	-
Fat content,%	3.85±0.063	3.70±0.176	3.81±0.109	3.87±0	3.55±0.050	3.76±0.149	-	-	-
Quantity of fat, kg	249.3±21.59	226.5±54.60	216.9±46.08	210.3±0	191.7±0.75	212.7±0.15	-	-	-
Protein content,%	3.16±0.086	3.12±0.056	3.25±0.092	3.30±0	3.03±0.025	3.25±0.033	-	-	-
Amount of protein, kg	202.9±13.88	186.7±45.26	184.8±36.71	179.3±0	163.3±1.69	181.8±36.96	-	-	-
(2 lactation)	12	9	13	1	2	3	-	-	-
Milk yield, kg	6851±1205	6824±1996	6502±1344	6358±0	6416±516	6051±764	-	-	-
Fat content,%	3.79±0.126	3.73±0.097	3.73±0.126	3.86±0	3.7±0.10	3.73±0.093	-	-	-
Quantity of fat, kg	259.0±43	252.9±69.94	241±44.13	245.4±0	264.0±0	224.9±22.32	-	-	-
Protein content,%	3.16±0.102	3.1±0.078	3.23±0.093	3.20±0	3.05±0.050	3.23±0.044	-	-	-
Amount of protein, kg	215.7±33.32	211.4±61.93	210.2±46.06	203.5±0	195.5±12.55	195.3±23.34	-	-	-
(3 lactation)	9	5	9	1	2	9	-	-	-
Milk yield, kg	7783±1742	6390±672	7239±1479	6601±0	6169±731	7648±137	-	-	-
Fat content,%	3.72±0.123	3.83±0.138*[a]	3.78±0.183	3.64±0	3.74±0.135	3.90±0.03	-	-	-
Quantity of fat, kg	290.8±67.65	244.9±30.25	275.9±60.92	240.3±0	229.4±18.97	298.2±3.05	-	-	-
Protein content,%	3.14±0.094	3.08±0.064	3.21±0.075	3.20±0	3.05±0.05	3.3±0	-	-	-
Amount of protein, kg	244.2±54.19	197.0±23.05	232.3±48.63	211.2±0	188.5±25.38	252.4±4.52	-	-	-
(lactation veins)	14	13	13	1	2	9	-	-	-
Milk yield, kg	8153±1210	7942 ±1380	7687±1092	6601±0	7211±2278	7637±148	-	-	-
Fat content,%	3.70±0.094	3.67±0.069	3.68±0.102	3.64±0	3.7±0.1	3.85±0.1	-	-	-
Quantity of fat, kg	301.6±43.07	292.3±52.45	282.6±38.79	240.3±0	266.5±3.09	293.9±5.72	-	-	-
Protein content,%	3.13±0.086	3.08±0.061	3.21±0.061	3.2±0	3.1±0.1	3.3±0	-	-	-
Amount of protein, kg	254.7±36.84	246.6±43.31	246.9±36.45	211.2±0	223.8±15.85	252±4.9	-	-	-

Significant: *=P<0.05; **=P<0.01; ***=P<0.001 (compared to animals of the third control group); a= P<0.05; b= P<0.01. (in comparison of animals of the first experimental group with analogues of the second experimental group) Mean values with different superscripts in the column differ significantly (p<0.05). TG5 thyroglobulin gene (TG-5); Ukrainian Red Dairy (URD); Ukrainian Black-speckled Dairy (UBSD); Ukrainian Red-speckled Dairy (URSD).

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Table 4 - Association of BLG gene SNPs with signs of cow dairy productivity									
Characteristic, key figure	Locus genotype ($\bar{X} \pm S_x$) in the breed section								
	AA			AB			BB		
	UBSD	URSD	URD	UBSD	URSD	URD	UBSD	URSD	URD
(1 lactation)	1	3	7	6	2	5	8	11	4
Milk yield, kg	7554±0	6681±1736	5884±1290	6663±413*[b]	4380±331	6014±842	6000±289	5805±123	4839±838
Fat content,%	3.7±0	3.72±0.147	3.81±0.118	3.88±0.04	3.80±0.045	3.82±0.102	3.84±0.062	3.67±0.2	3.76±0.15
Quantity of fat, kg	279.5±0	248.5±65	225.1±52.97	262.7±11.21	166±11	230.7±37.67	230.6±12.71	218.7±51.33	182.4±33.9
Protein content,%	3.20±0	3.03±0.044	3.24±0.089	3.16±0.108	3.20±0	3.3±0.058	3.17±0.081	3.12±0.053	3.21±0.106
Amount of protein, kg	241.7±0	203.6±56.21	190.2±40.03	210.1±8.13	140.2±10.61	198.5±27.69	189.8±6.66	181.1±40.17	156.2±29.40
(2 lactation)	1	2	7	5	2	5	7	8	4
Milk yield, kg	4722±0	7140±1240	6123±1373	6614±609	5475±1778	6727±880	7255±1277	7034±380	6549±1220
Fat content,%	3.75±0	3.72±0.115	3.77±0.109	3.77±0.097	3.70±0.13	3.63±0.135	3.82±0.145	3.71±0.092	3.77±0.082
Quantity of fat, kg	177.1±0	292.5±28.5	229.8±45.32	248.9±22.42	200.5±58.47	243.8±30.89	275.9±45.34	259.6±59.19	245.2±39.23
Protein content,%	3.20±0	3.05±0.05	3.21±0.088	3.17±0.136	3.23±0.075	3.29±0.05	3.16±0.078	3.08±0.063	3.19±0.095
Amount of protein, kg	151.1±0	217.2±34.27	196.5±43.6	208.8±15.56	175.2±53.23	221.2±28.57	228.0±35.77	217.1±53.92	209.3±41.9
(3 lactation)	1	2	5	4	2	3	5	4	3
Milk yield, kg	11046±0	6564±336	7304±1744	6201±1457	7424±0	6970±1483	8161±896*	5934±779**[a]	7671±282.4
Fat content,%	3.91±0	3.64±0.04	3.87±0.218	3.67±0.08	3.94±0	3.76±0.087	3.71±0.134	3.85±0.106	3.73±0.167
Quantity of fat, kg	431.9±0	238.8±9.6	287.4±84.58	228.2±55.94	292.5±0	261,9±55.33	302.5±35.24	228.3±26.82	285.8±10.35
Protein content,%	3.1±0	3.05±0.05	3.25±0.055	3.18±0.088	3.2±0	3.26±0.011	3.14±0.088	3.05±0.05	3.15±0.1
Amount of protein, kg	342.4±0	200.4±13.53	237.5±56.71	197.2±47.29	237.6±0	227.5±48.56	255.5±25.58	180.9±23.89	241.9±16.32
(lactation veins)	1	3	7	6	2	5	8	11	4
Milk yield, kg	11046±0	7668±1078	6947±1182**[a]	7376±820	8338±1085	8303±478	8181±1119	7750±1292	8174±407
Fat content,%	3.91±0	3.67±0.047	3.76±0.135	3.75±0.048	3.65±0.075	3.71±0.086	3.63±0.059	3.68±0.083	3.65±0.111
Quantity of fat, kg	431.9±0	281.7±43.09	260.4±45.36	276.6±28.39	304.7±45.82	308±19.41	296.4±38.36	285.2±48.01	297.9±14.52
Protein content,%	3.1±0	3.10±0.067	3.24±0.055	3.16±0.108	3.08±0.075	3.25±0.06	3.11±0.058	3.09±0.062	3.19±0.095
Amount of protein, kg	342.4±0	238.1±34.19	224.8±40.02	232.8±26.63	255.6±27.13	269.6±12.46	253.4±32.16	241±42.13	260.9±16.08

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

Table 5 - Association of Pit-1 gene SNPs with signs of dairy productivity of cows

Characteristic, key figure	Locus genotype ($\bar{X} \pm S_x$) in the breed section								
	AA			AB			BB		
	UBSD	URSD	URD	UBSD	URSD	URD	UBSD	URSD	URD
(1 lactation)	1	3	0	10	9	10	4	3	6
Milk yield, kg	6117±0	5690±652	-	6293±543	6202±1630	5431±1013	6622±633*[b]	5238±947	6050±1297
Fat content,%	3.83±0	3.84±0.058	-	3.86±0.057	3.63±0.211	3.77±0.134	3.83±0.065	3.69±0.127	3.85±0.085
Quantity of fat, kg	234.3±0	218.7±27.78	-	243.0±22.56	232.3±3.11	206±43.1	259.1±17.1	193.6±35.14	233.2±52.46
Protein content,%	3.20±0	3.12±0.078	-	3.15±0.11	3.11±0.051	3.22±0.098	3.20±0.025	3.1±0.067	3.30±0.077
Amount of protein, kg	195.7±0	177.1±19.15	-	197.7±12.59	192.7±51.79	175.4±32.42	211.8±18.61	162.7±29.56	199.1±41.16
(2 lactation)	1	2	-	9	6	10	3	3	6
Milk yield, kg	7732±0	4804±1107	-	7116±1176	7149±1656	6394±1002	5602±638.9	7251±1526*[b]	6458±1663
Fat content,%	4.0±0	3.81±0.025	-	3.76±0.131	3.72±0.107	3.69±0.13	3.82±0.071	3.68±0.078	3.79±0.102
Quantity of fat, kg	309.3±0	182.5±40.5	-	266.6±39.35	264.5±59.51	234.7±28	214.7±28.72	284±42.82	243.5±59.34
Protein content,%	3.2±0	3.15±0.15	-	3.14±0.11	3.06±0.061	3.20±0.094	3.23±0.044	3.12±0.022	3.28±0.069
Amount of protein, kg	247.4±0	149.7±27.66	-	222.2±31.38	219.2±52.82	205.3±35.87	181.4±23.36	226.4±49.45	211.0±52.53
(3 lactation)	1	2	-	6	3	7	3	2	4
Milk yield, kg	8489±0	6972±452	-	7556±1518	5512±477	7934±1026	7610±2290**	6903±350	6227±992
Fat content,%	4.0±0	3.79±0.15	-	3.64±0.07	3.83±0.102	3.89±0.135	3.76±0.1	3.78±0.175	3.65±0.06
Quantity of fat, kg	339.6±0	264.9±27.59	-	274.9±54.05	210.7±12.34	310.4±51.6	289.4±94.97	260.6±12.21	226.7±35.68
Protein content,%	3.1±0	3.1±0.1	-	3.17±0.111	3.03±0.044	3.20±0.095	3.13±0.044	3.1±0	3.29±0.015
Amount of protein, kg	263.2±0	216.6±20.98	-	238.9±47.43	167.0±13.23	253.9±35.04	237.3±70.08	214±0.11	204.6±32.82
(lactation veins)	1	3	-	10	9	10	4	3	6
Milk yield, kg	8489±0	7493±1287	-	7918±1300	7557±1160	7689±790	8270±1388	9061±1047	7659±1122
Fat content,%	4.0±0	3.74±0.076	-	3.65±0.067	3.66±0.069	3.73±0.136	3.82±0.044	3.68±0.069	3.69±0.089
Quantity of fat, kg	339.6±0	279.8±47.16	-	288.3±44.48	276.9±43.8	285.8±25.45	316.9±57.52	333.8±42.77	282.8±45.2
Protein content,%	3.1±0	3.05±0.067	-	3.11±0.086	3.09±0.053	3.22±0.076	3.19±0.063	3.12±0.078	3.25±0.05
Amount of protein, kg	263.2±0	228.1±36.44	-	245.1±38.07	234.8±39.83	247.3±27.02	263.7±44.87	281.6±27.94	248.8±36.6

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

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CONCLUSION

1. Indicators of all groups of cows by the locus of the leptin gene, which were included in the study, for the first, second third and higher lactation tended to increase their productivity, which is explained by their physiological maturity and the development of productive qualities with age. And in the context of experimental groups, heterozygous genotypes of CT turned out to be the best.

2. The relationship between the economically useful signs of cows and their genotype by the capa-casein gene has been proven. Namely, animals with homozygous genotype AA were characterized by higher manifestations of milk productivity in terms of utilization, fat and protein content, as well as their amount in milk, compared to other genotypes, both in the context of experimental breeds and in age dynamics. But the best raw suitability will be characterized by milk obtained from cows with the BB genotype.

3. The influence of homo- or heterozygosity of the thyroglobulin gene on most productive features of cows, both in the section of breeds and in age dynamics, has been established. Cows carrying the homozygous CC genotype were characterized by higher values of milk productivity in the section of the first, second, third and highest lactation, regardless of the breed affiliation of the cows. At the same time, the second homozygous genotype of TT was not found in any of the studied breeds of UBSD, URSD and URD, which, in our opinion, is associated with the individual characteristics of these breeds and their origin.

4. It was determined that the effect of the homo- or heterozygous state of BLG globulin on signs of milk productivity is less noticeable. Thus, cows of UBSD (II lactation), URSD (III lactation) with genotype AA, and a slightly large representative of the first group with genotype AA alternately during ontogenesis, with the exception of II lactation, were characterized by somewhat reduced fatigue. In general, homo- and heterozygous genotypes of VHMP and UKM of rocks of different combinations were observed with higher productivity values.

5. It has been confirmed that the pituitary gene of specific Pit-1 factor was associated with high-yield animals with homozygous AA and BB genotypes for all lactation among UBSD and URSD livestock, while URD cows had the best BB genotypes, which also causes the largest amount of milk fat in these animals. But the amount of fat does not differ significantly between homo- and heterozygous genotypes.

In this way the results obtained can be used in the practical breeding work of breeding and livestock farms using traditional methods of selection, monitoring and maintaining the genetic diversity of specific herds at an optimal level. This will ultimately provide an increase in the productivity of cattle due to the introduction into reproduction of animals with more desirable genes for capacasein, thyroglobulin, leptin, pituitary transcription factor and beta-lactoglobulin.

DECLARATIONS

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Authors' contribution
G. Julia and G.Mikhail participated in the design of the study, conducted experiments and wrote the original manuscript. G. Mikhail and K. Elena helped in ordering the data and calculations. G. Julia and K. Elena critically edited the manuscript. All authors read and agreed with the published version of the manuscript.

Conflict of interests
The authors did not declare any conflict of interest.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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EFFECT OF FEEDING SAGE MEAL TO WEANED AWASSI MALE LAMBS ON BODY PERFORMANCE AND MEAT QUALITY

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ABSTRACT: An experiment was conducted for 8 weeks on 15 Awassi lambs using sage dry meal. Animals were randomly allocated into 3 groups: control group meal (CGM: 0% sage; *Salvia officinalis*), experimental group meal 1 (EGM1: 1% sage) and experimental group meal 3 (EGM3: 3% sage) by 5 heads. Feed intake at the 8th week increased proportionally with live body weight (LBW). Cumulative live body weight gain increased slightly more in EGM3 after the 5th week to attain 10.7 Kg at 8th week. Feed conversion ratio (FCR) was most effective and attained 3.83 ± 0.97 in EGM3 vs 4.14 ± 0.53 and 4.15 ± 0.64 in CGM and EGM1, respectively. After cooling, luminance ranged between 45.41 ± 2.97 in CGM and 47.28 ± 5.63 in EGM1 whereas in EGM3 it was 47.28 ± 5.63. Redness, a*, after cooling was lowest in EGM3 (20.15 ± 3.29) followed by CGM (22.61 ± 3.41) and EGM1 (24.97 ± 1.24). Yellowness b* after 1 month of freezing is positively correlated with the achieved results after 24 h of cooling. The least losses in water after cooling was in EGM3 attaining 11.39 ± 2.39%. Meat of CGM loses more water after cooking (30.30 ± 6.52%) than other groups. Furthermore, after 24 hours of cooling, the most tender meat was in EGM1 (4.87 ± 0.44mm) in comparison to CGM (3.3 ± 0.64mm), whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 ± 0.8mm). It is concluded and recommended to use rations containing sage meal in the daily feeding of Awassi sheep.

Keywords: Awassi lambs, Feeding, Meat quality, Performance, Sage dry meal.

INTRODUCTION

The productivity loss in small ruminant farming is a significant issue, and food scarcity, which is primarily brought on by high costs, has been highlighted as one of its primary reasons. The maximization of low concentrate feed costs while ensuring an increased net yield are major issues in such agricultural enterprises (De Roest et al., 2018; Hanrahan et al., 2018). This topic has been the subject of a great deal of recent research (Jakubowska and Karamucki, 2021; Jordán et al., 2020), and different introduction tactics have been examined. Antibiotics are a substance that has received harsh criticism (Oberoi et al., 2019). The use of antibiotics is restricted for a number of significant reasons, two of which being the development of drug resistance in bacteria and the presence of drug residues in meat (Bacanli and Başaran, 2019; Patel et al., 2019).

Antibiotics have been removed from the diet, which has led to subpar performance and an increase in illness susceptibility. To get over these difficulties, efforts were undertaken to develop different solutions. Use of growth boosters with a natural origin is one technique that has garnered a lot of interest lately. Strong evidence suggests that herbs, spices, and their products have antioxidative, antibacterial, and growth-promoting benefits on animals, according to Odoemelam et al. (2013). It is possible that some herbs and spices have antioxidant properties that protect both the quality of feed and food made from animals fed these substances (Odoemelam et al., 2013). Aromatic herbs have been employed as food preservatives and in traditional medicine since ancient times (Christaki et al., 2020). The Mediterranean region is the origin of many of the most well-known fragrant plants, including oregano, rosemary, chamomile, sage, anise, basil, etc. Because they are organic, natural, and usually regarded as safe products, the demand for these plants and the derivatives they produce has recently surged. As a result, aromatic plants and their extracts have the potential to lead to new developments in food and health goods for both humans and animals (Giannenas et al., 2020; Christaki et al., 2020). The use of antibiotic growth promoters as a feed additive has been prohibited since the turn of the century, according to Rahman et al. (2022), Wang et al. (2020) and Nm et al. (2018). These antibiotics have been included in animal feed rations to promote the growth of beneficial microorganisms in the intestinal microflora and to avoid disease. Scientists began seeking for alternatives once most antibiotic growth promoters were banned. They concentrated on using herbs, spices, and plant extracts (essential oils—EO) as potential antibiotic substitutes as a result. They increased feed intake, feed conversion ratio, and carcass yield activity when added to the feed ration or water (Patel

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et al., 2015). Sage is one of the fragrant plants that can be highlighted sage (*Salvia officinalis*). According to Caesar and Cech (2019) sage's bioactive components work together synergistically to exert pharmacodynamic effects that are mostly salt secretive, antiseptic, antidiarrheal, anti-inflammatory, immuno-stimulating, and analgesic.

This study seeks to explore the effects of substituting sage dry meal for antibiotics and antioxidants in basic rations when supplementing sheep for this purpose. Sage dry meal contains natural phytogenic substances at varied quantities. In comparison to the control group of antibiotic-free lambs, the body performance and meat quality of groups of Awassi male lambs are noted.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Bioethics Committees of the Lebanese University, Faculty of Agriculture, Department of Animal Production, and the University of Forestry, Sofia, Bulgaria. and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of November 24, 1986.

Animals and study field

A total of fifteen Awassi lambs, sixty to seventy days old, were purchased from Bekaa valley in Lebanon and divided into three groups of five animals each. The site was designed to study the impact of feeding male sheep different supplementation levels of sage meal on body performance and meat quality during the fattening period. Conditions included availability of ambient temperature of 25°C, water and feed were offered *ad libidum* for the duration preceding the experimental period.

Methods

At the start of the experiment, all animals were fed a basic ration (BR) for 5 days after which sage was added after allotting them into 3 groups, of 5 lambs each. Animals of the control group (CGM) were fed free choice antibiotic-free and antioxidant-free basal diet in mashed form based on yellow corn-soybean meal mixture and hay. The remaining 2 groups were fed the following experimental rations: Animals of group (EGM1) were fed BR supplemented with 1% sage dry meal and EGM3 had BR with 3% sage dry meal. All experimental rations fed to animals were not exceeding 18% crude protein and 2718 ME kcal/kg of feeds. Daily observations of the lambs revealed no signs of illness. Every week, a live body weight (LBW) scale was used to measure each participant (kg). On a weekly basis and at slaughter, feed conversion rate (FCR) and live body weight growth (LBWG) were calculated (after 8 weeks of the experiment). After 24 hours of cooling and one month of deep freezing, the physical characteristics of the meat were examined (post mortem), and the L*, a*, and b* colours as well as the pH level were measured using a chromometer and pH-meter in accordance with Offer and Knight's procedure (1988). Measurements were made on the surface of the meat, free of any visible colour flaws, three times each sample (bruises, blood spots, and haemorrhages). According to Honikel (1998), cooking loss was evaluated, thawing loss was calculated, and hardness (tenderness) was estimated using a penetrometer.

Statistical analysis

Using the "SigmaStat software V. 3.5," one way analysis of variance (ANOVA) of the results was used to assess the statistical differences between the treatments. Results were shown as Mean values with standard deviation. The significance level was set at P 0.05. With different confidence levels as P0.001***, P0.01**, and P0.05*, Pearson Correlation was used to examine the positive and negative independence of results of the various variables among all experimental groups.

RESULTS AND DISCUSSION

Table 1 shows the amount of the cumulative feeds consumed (cFI, kg/group) and Figure 1 estimated cumulative feed intake (cFI, kg/head) by animals of all groups during the eight weeks of the trial. Both Figure 1 and Table 1 revealed an equal intake of feeds distributed on the experimental animals. The results obtained demonstrate that the overall feed intake at the end of 8th week of the trial increased proportionally with the increase of LBW, attaining a small increase ($P>0.05$) in group EG3 (197.5 kg/group) by 3.9 % and EGM1 (192.5 kg/head) by 1.3 % in comparison with the negative control group (CG) where it consumed the amount of 190 kg/group.

The observation of animal during the experiment showed that animal finish taking the ration containing sage faster than the control group, this due to the beneficial effects of sage in animal nutrition may include the stimulation of appetite and feed intake. This result agrees with the findings obtained by the experiment done before. This result agrees with the findings obtained by Kamel (2001), who applied essential oils of different herbs and spices in animal feeding resulting in increased feed intake in comparison with rations exclusive of any herb supplementations. In addition, this improvement in feed consumption was observed may be due to the appetizing effect of active ingredient (borneol) in chamomile having anti-inflammatory, antiseptic, diaphoretic and sedative properties (Srivastava et al., 2019) by killing and inhibiting the harmful intestinal microorganisms in the intestinal tract of the animals (Nazarizadeh et al., 2019).

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Table 1 - Estimation of the overall Feed Intake (FI) among experimental groups of Males, kg/group

At the end of week	BR + commercial antioxidants + antibiotics + vitamin premix + mineral premix	1% *Salvia officinalis* to BR + vitamin premix + mineral premix	3% *Salvia officinalis* to BR + vitamin premix + mineral premix
	CGM	EGM1	EGM3
1st	20.00	20.00	20.00
2nd	40.00	40.00	40.00
3rd	65.00	65.00	65.00
4th	90.00	90.00	90.00
5th	115.00	115.00	115.00
6th	142.50	142.50	142.50
7th	170.00	170.00	170.00
8th	190.00	192.50	197.50

BR: basic ration, CGM: control group meal, EGM1: experimental group meal 1, EGM3: experimental group meal 3

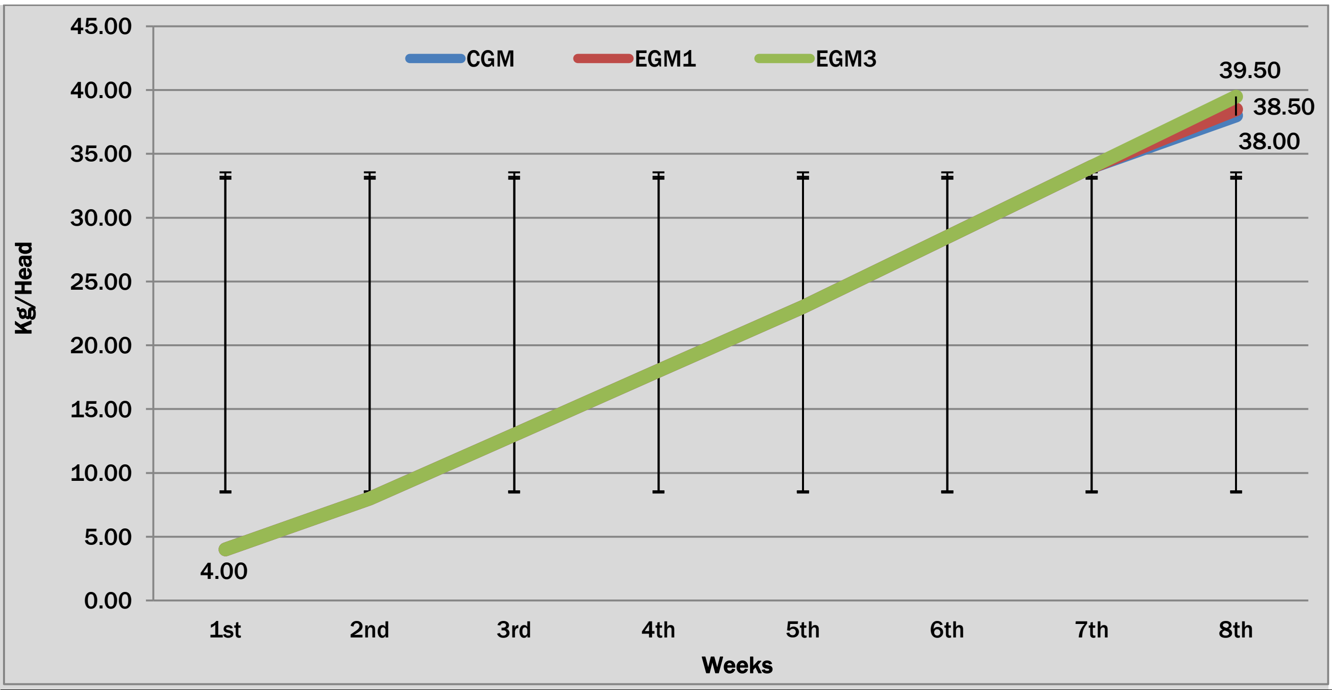

Figure 1 - Estimation of weekly-cumulative feed intake among experimental groups of males, kg/head

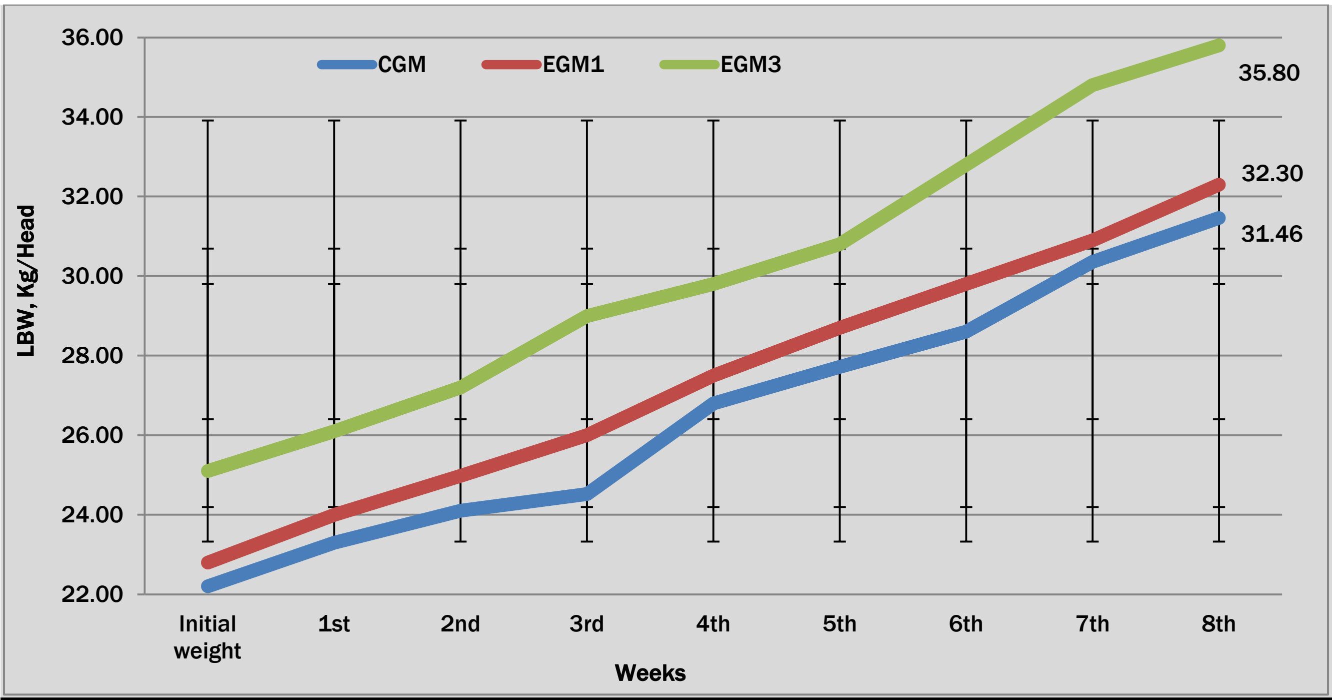

Figure 2 - Live body weight among experimental groups of Males, kg/head. (There was a difference (P<0.05) at the end of the 7th week between the 3rd and the 2 other groups with a slight increase in LBW for the 3rd group).

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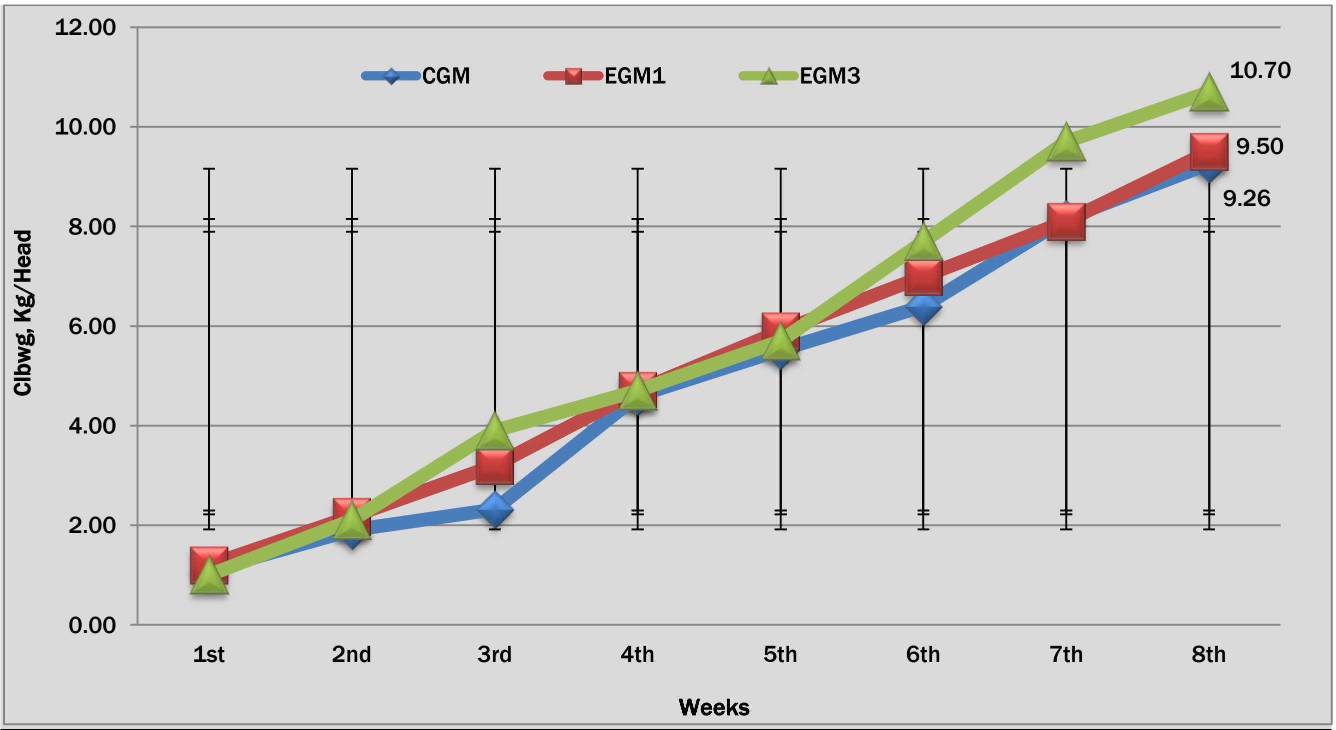

Figure 3 - Weekly-cumulative Live body weight gain variations among experimental groups of Males, kg/head. At weeks 6th and 7th EGM3 attained higher (P<0.05) LBWG levels (2 ± 0.93 kg) among all groups where it decreased later (P>0.05) in the last week (1 ± 0.00 kg) of the trial.

The results on LBW of the experimental groups where the effect of *Salvia officinalis* in free-antioxidant basic ration is studied are summarized in Figure 2. The results showed that there is no significant difference (P>0.05) between the 3 groups during the first 6 weeks. Meanwhile, there was a difference (P<0.05) that appeared at the end of the 7th week between the 3rd and the 2 other groups with a slight increase in LBW for the 3rd group (34.8 ± 3.15 kg vs 30.36 ± 1.6 and 30.90 ± 2.64 kg in groups CGM or control) and CGM1 or 1% sage), respectively. This difference (P<0.05) continued to show up in the 8th week between the 1st (31.46 ± 1.29 kg), 2nd (32.30 ± 2.16) and 3rd (35.80 ± 3.15 kg) groups attaining higher LBW levels than the control (CGM). These results showed that introducing *Salvia officinalis* to the ration has increase the LBW of sheep slightly more than the control group with no significant difference between the 3 groups these results agreed with the other experiment done before. Another study was conducted by LenuŃa and Leonte (2015) to determine the effect of sage essential oil vs antibiotics. They reported a best average body weight at broilers fed 8% sage (E3 group), which at the age of 42 days had an average weight of 2919 g, with the best daily live weight of 69, 50 g. Compared to the key group, the other groups treated with various levels of sage oil had daily average intakes higher with 1% (E1), 2% (E2) and 8% (E3) (P<0.05). The results obtained by LenuŃa and Leonte (2015) agrees with the study conducted by Windisch et al. (2009) revealing that feed additives derived from plants, also called phytogenics or phytobiotics or botanicals, can be included in animals' diets to improve their productivity including live body weight. This is also in support with the results obtained by Kolacz et al. (1997) that showed a significant improvement of body weight (BW) due to the main constituents of the herbs; the supplementation of 2% chamomile flowers dry meals plays a role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction in the animal body (Zhian, 2013).

The weekly variation in the average LBWG, kg/head is shown in Figure 3, so that at the end of 1st week group, diet supplemented with 3% of *Salvia officinalis* in the basic ration (EGM3) has the least (P>0.05) weekly LBWG (1 ± 0.7 kg) in comparison with 1st (1.1 ± 0.65 kg) and 2nd (1.2 ± 0.44 kg) groups. Whereas, at weeks 6th and 7th, EGM3 attained higher (P<0.05) LBWG levels (2 ± 0.93 kg) among all groups where it decreased later (P>0.05) in the last week (1 ± 0 kg) of the trial. The group (EGM1) with 1% of *Salvia officinalis* had a fixed rate of weekly LBWG, whereas control group (CGM) and 3rd group (EGM3) followed an irregular weekly rate of LBWG showing a low LBWG at 3rd week, followed by high LBWG at 4th and 7th weeks (Figure 3).

The weekly cumulative live body weight (wcLBWG) increased equally in the different groups but slightly more in EGM3 after the 5th week to attain 10.7 Kg at 8th week (P>0.05). This suggests that the use of *Salvia officinalis* in the diets of ruminants may modulate ruminal fermentation by reduction of methane production, thus potentially involving productive and environmental benefits; however, in vitro dry matter digestibility was decreased linearly. And we should notice that the temperature at the 8th week was more than 45 °C, and this might be the reason in the reduction of feed intake causing stress to animals. And as result a decrease in the BWG at this week to the 2 group with *Salvia officinalis* in the ration. Abd El-Maksoud et al. (2002) observed that the highest weight gain of Nile tilapia (*Oreochromis niloticus)* fingerlings was obtained when fed with 3% marjoram leaves of the total diet. This also resulted in the best protein and

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energy utilizations apart from having a significant effect on body composition. On the contrary, results obtained by Abd El-Maksoud et al. (2002) demonstrated that Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets contained 0.5-1% of chamomile flowers, Nigel seed or marjoram leaves alone showed lower performance than the control group. In my study the results showed that the LBWG is lower in the 2 groups (IGM1-IGM3) with *S. officanalis* have lower BWG than the control group (CGM) which agree with results of Abd El-Maksoud et al. (2002).

Table 2 showed that there was no significant difference (P>0.05) among animals all in the Weekly-cumulative FCR of the three experimental groups during all the period of the experiment. As it is shown from Table 2 that FCR of EGM3 (5.9 ± 3.56) at the end of the 1st week was insignificantly (P>0.05) higher than the other 2 groups (CGM = 4.89 ± 2.79 and EGM1 = 3.61 ± 0.96). Nevertheless, this indicator attained the lowest level and most effective conversion of feeds into body gain attaining 3.83 ± 0.97 in animal group EGM3 Vs 4.14 ± 0.53 and 4.15 ± 0.64 in groups CGM and EGM1, respectively. Similarly, Agarwal et al. (2009) demonstrated that plant extracts may decrease the digestibility of feeds because of the inhibition of cellulolytic bacteria metabolism. Moreover, Patra et al. (2006) highlighted that the inhibitory effects of plant extracts on rumen cellulolytic bacteria may cause a decrease in feed digestibility. On the other hand, the supplement increased the total bacteria population even by 23.4%, which can be explained by ecological succession. Inhibition of the protozoa population probably induced a new ecological niche for bacteria.

Table 2 - Weekly-cumulative FCR variations among experimental groups of Males

Diet / Time	BR + commercial antioxidants + antibiotics + vitamin premix + mineral premix	1% *S. officinalis* to BR + vitamin premix + mineral premix	3% *S. officinalis* to BR + vitamin premix + mineral premix
At the end of week	**CGM**	**EGM1**	**EGM3**
1st	4.89±2.79	3.61±0.96	5.90±3.56
2nd	5.22±2.45	4.06±1.40	4.97±2.78
3rd	6.44±2.55	4.69±1.98	3.65±1.52
4th	4.06±0.80	4.04±1.00	4.19±1.75
5th	4.28±0.81	4.28±1.33	4.29±1.48
6th	4.49±0.53	4.34±1.13	3.90±1.13
7th	4.24±0.65	4.37±0.89	3.67±1.02
8th	4.14±0.53	4.15±0.64	3.83±0.97

BR: basic ration, CGM: control group meal, EGM1: experimental group meal 1, EGM3: experimental group meal 3.

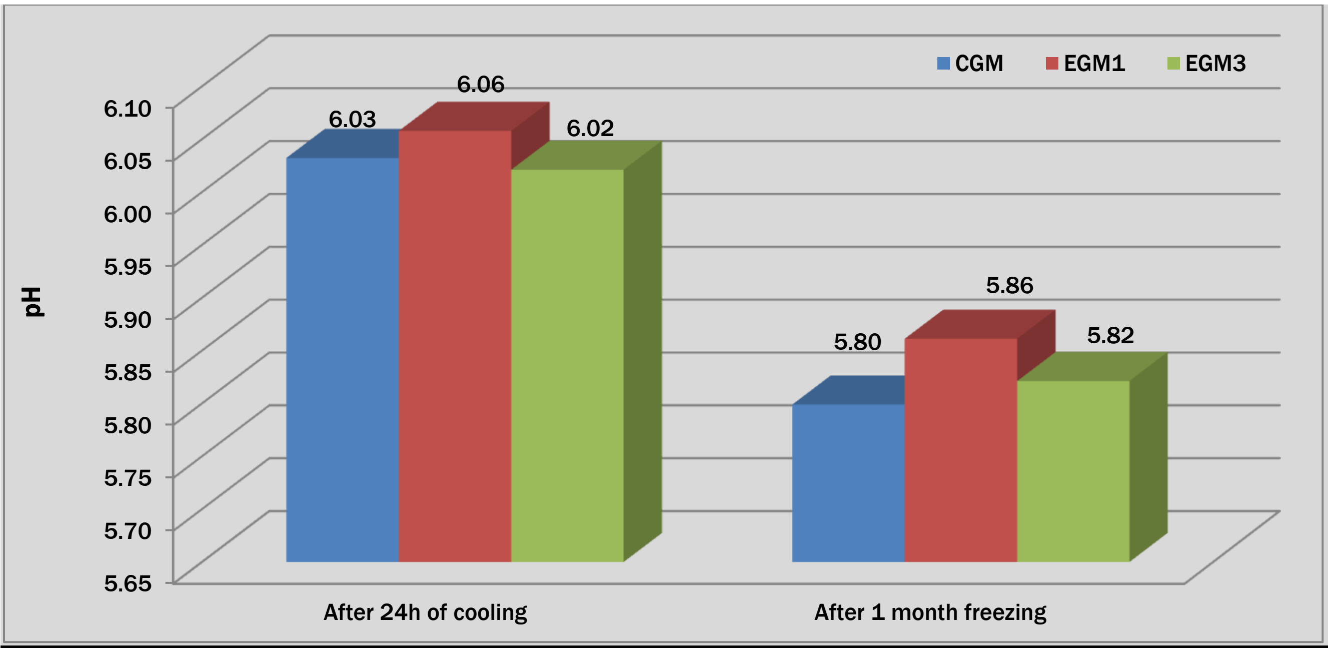

Figure 4 - pH variations before and after freezing of mutton.

Figure 4 summarizes the variations in pH results. pH of meat is related to glycogen level in muscle tissues. The results of pH level where it calibrated insignificantly (P>0.05) after 24 hours of cooling at 5-7 °C between 6.03 ± 0.10 in group CGM and 6.02 ± 0.28 in group EGM3 and decreased after 1 month of freezing to calibrate insignificantly (P>0.05) between 5.8 ± 0.04 and 5.82 ± 0.07 in CGM and EGM3, respectively. More basic insignificant values (P>0.05) were obtained in EGM1 group before (6.06 ± 0.21) and after freezing (5.86 ± 0.10) whose animals were fed a supplementation

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of 1 % *Salvia officinalis* to the basic ration of sheep. Figure 4 shows that pH of meat at 24h of cooling after slaughter is more basic than the pH at 1month of freezing where after this duration of time pH of meat becomes more acidic and this due to maturation of meat where with maturation the meat becomes more acidic. Moreover, the effect of sage EO *in vivo* could be significantly different than that reported *in vitro*. This difference could be related to the pH in the media where the oil is supposed to exert its effects. For example, the pH of milk varies between 6.4 and 6.6, but in the case of an infection, it increases to pH of 7.4 (Ziv, 1980). For best results, lower pH such (5-6) is preferred for sage EO (Gutierrez et al., 2008).

Color of meat is affected by the interaction of myoglobin pigment in meat with the absorbance and reflectance of light (AMSA, 2012). Figure 5 showed the obtained results (P>0.05) of *L* after slaughter (cooling and freezing). Luminance (L*) of meat reveals the reflection of water on the surface of meat samples. After 24 h of cooling Luminance *L* ranged insignificantly (P>0.05) between 45.41 ± 2.97 in CGM and 47.28 ± 5.63 in group EGM1 whereas in group EGM3 it was 47.28 ± 5.63, lower than control. This tendency was lowered after 1 month of freezing in CGM (42.75 ± 2.34) and EGM1 (42.94 ± 2.57) and higher in EGM3 (42.16 ± 0.67). The polyphenols contained in *Salvia officinalis* are likely to be oxidized to corresponding quinines by polyphenol oxidases, which are widespread in plant materials. Such quinines may condense to form darkened which results in an intense color of meat (Liu et al., 2009).

Figure 6 demonstrates the variation of the results after cooling and freezing of different groups. The redness depends on the type of fiber in muscle and the presence of myoglobulin in tissue where iron is abundant. There is high difference in redness in the results obtained before and after freezing among all groups (P>0.05). The lowest level of *a* before after cooling was seen in EGM3 (20.15 ± 3.29) followed by CGM (22.61 ± 3.41) and EGM1 (24.97 ± 1.24). This sequence was continued for after freezing for groups CGM (21.83 ± 6.22) and EGM1 (22.43 ± 3.03) but not for EGM3 where this indicator was higher (22.99 ± 4.27) during this physical state. It was noted that meat color a* was negatively correlated (P<0.05) with thawing loss (%) after 1 month of freezing (Figure 6). Addition of natural antioxidants may improve oxidative stability of meat what can be the reason for smaller changes in meat color (Hanczakowska et al., 2015).

Yellowness refers mainly to the intramuscular fat tissues. Figure 7 shows the level of *b* during the whole trial. The results showed that yellowness increased before and after freezing and the difference is statistically insignificant (P>0.05). The highest effect was shown in EGM1 (14.03 ± 4.88) followed by CGM (11.90 ± 4.47), whereas the lowest was attained in EGM3 (7.84 ± 1.38). The same tendency was followed by the same manner during the state of after freezing but on lower levels (15.03 ± 2.04, 14.72 ± 1.82 and 13.63 ± 2.00), respectively. The results obtained showed that b* after 1 month of freezing was positively correlated (P<0.05) with the achieved results after 24 h of cooling (Figure 7). According to the mentioned results groups was characterized as samples with insignificant color changes and the observer perceived a clear similar color. It means that observer can perceive one color only in all simple. This obtained result agrees with the study conducted by Hanczakowska et al. (2015) revealing that addition of natural antioxidants may improve oxidative stability of meat what can be the reason for smaller changes in meat color.

Drip loss is the leakage of microfibers along with increased loss of water, nutrients and proteins from meat. The drip and thawing losses are due to the breakdown of the cell membrane and the diffusion of water outside the cell. Figure 8 illustrates the variation in drip and thawing losses among the three groups (P>0.05). Figure 8 showed the least losses in water after cooling in EGM3 followed by CGM and EGM3 attaining the levels of 11.39 ± 2.39%, 15.97 ± 10.73 and 11.39 ± 2.39%, respectively. Consequently, the loss in the thawing water after freezing was lower than the obtained drip loss but in a reversed order whereas the lower was in EGM1, followed by CGM and EGM3 (2.22 ± 0.28%, 2.54 ± 0.26 and 3.57 ± 1.68%), respectively. These results are insignificantly different between groups after freezing and either after cooling, the drip loss is high after cooling or thawing loss is relatively low after freezing.

Cooking loss is the amount of water lost due to the cooking process. The result of cooking loss in Figure 9 shows that the cooking loss after freezing was lower in all groups if to compare to the obtained results attained after cooling P>0.05). It is worth to note that meat of CGM loses more water after cooking than EGM3 and EGM1 (30.30 ± 6.52%, 29.40 ± 2.06 and 27.79 ± 1.02%), respectively. The cooking loss after freezing resulted in the same sequence where the lowest was attained in EGM1 (25.81 ± 1.58%). This decrease in weight or the high cooking loss was very natural due to the decrease of water holding capacity by the effect of proteins' denaturation.

Tenderness level depends on the level of maturation of muscle. Figure 10 shows the variation in penetration levels after using Penetrometer (P>0.05). Results showed that after 24 h of cooling the most tender meat after cooking was obtained from animal group EGM1 (4.87 ± 0.44mm) fed 1% *S. officinalis* with the basic ration in comparison to the animals of control group CGM (3.3 ± 0.64mm) fed no *S. officinalis*, whereas EGM3 occupied the 1st place in cooked meat tenderness after 1 month of freezing (5.4 ± 0.8mm) followed by both CGM and EGM1 (4 ± 0.63mm).

It was noted that meat tenderness was negatively correlated (P<0.01) with b* results after 24 h of cooling and 1 month of freezing (Figure 10).

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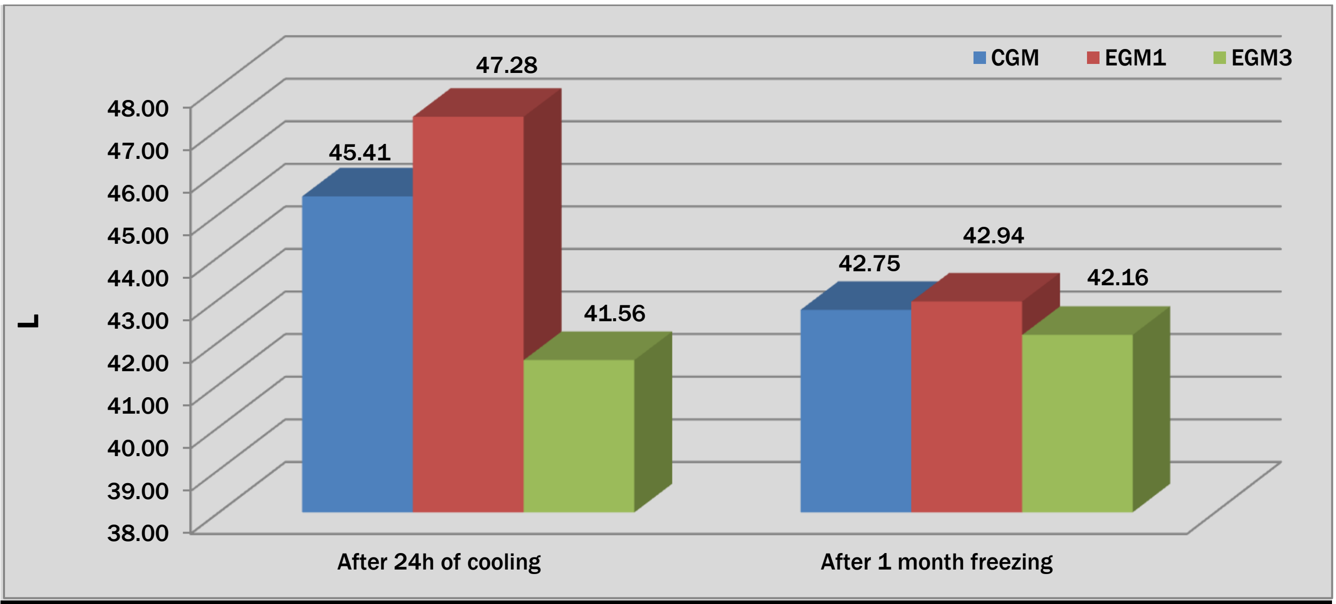

Figure 5 - *L* color variations before and after freezing of mutton

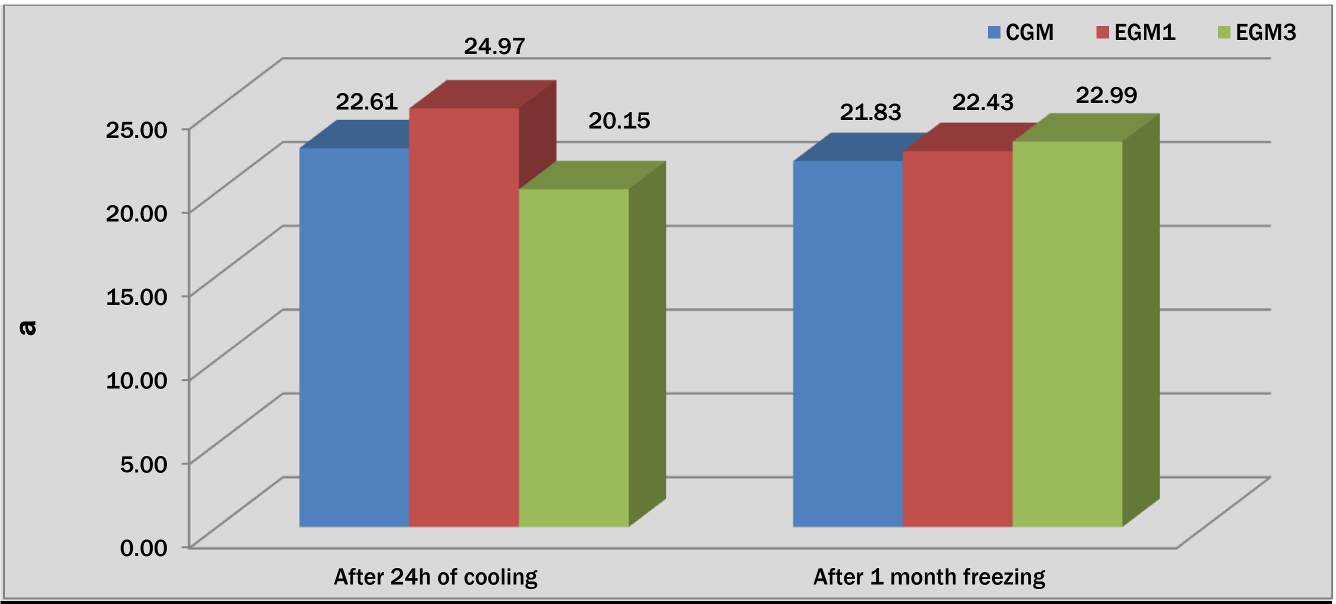

Figure 6 - *a* color variations before and after freezing of mutton. (Meat color a* was negatively correlated (P<0.05) with thawing loss (%) after 1 month of freezing).

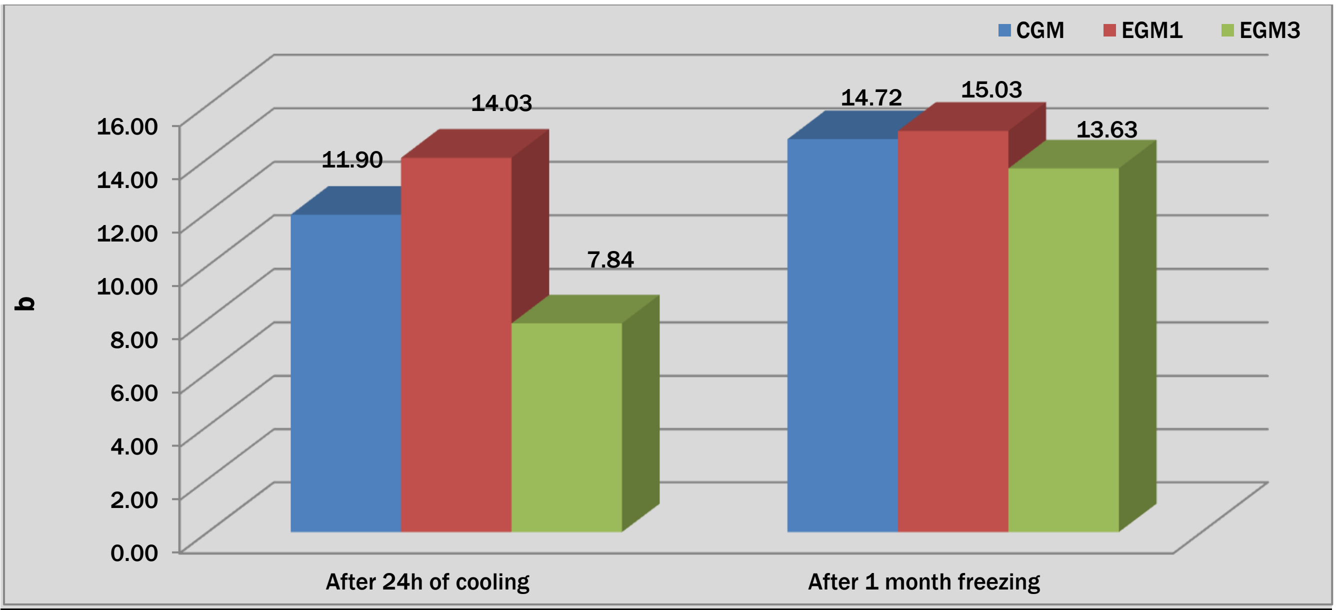

Figure 7 - *b* color variations before and after freezing of mutton. (*b* color value or yellowness after 1 month of freezing was positively correlated (P<0.05) with the achieved results after 24 h of cooling).

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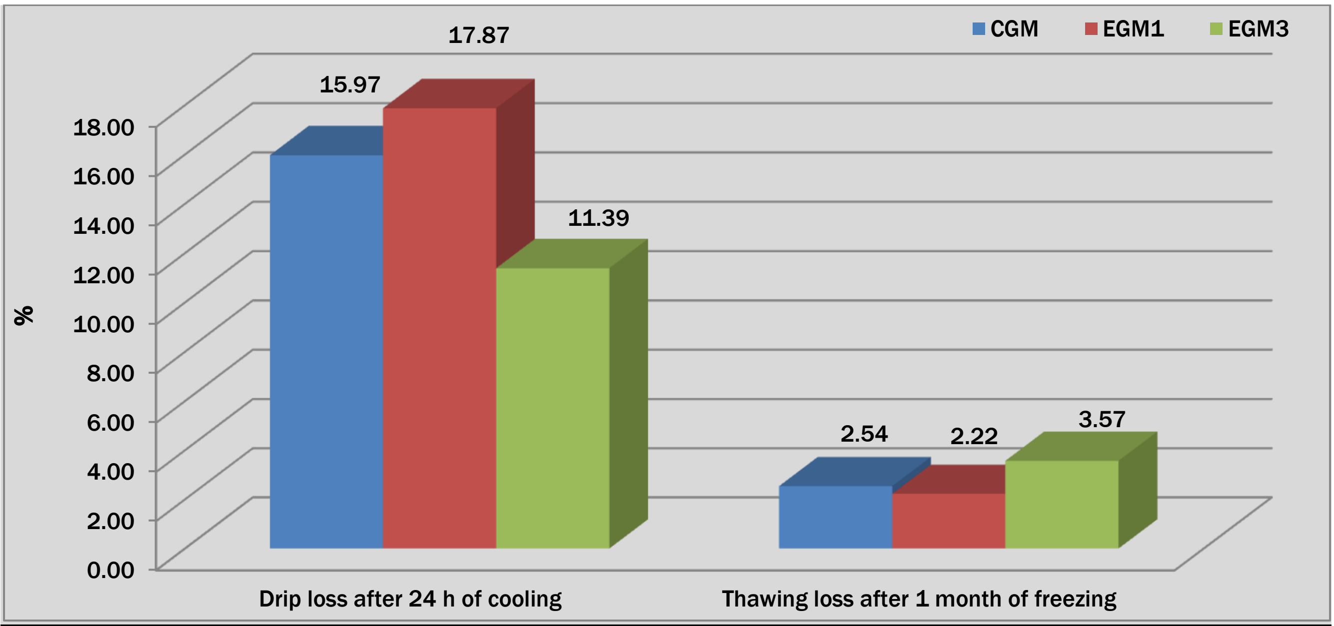

Figure 8 - Drip and Thawing losses before and after freezing of mutton (%).

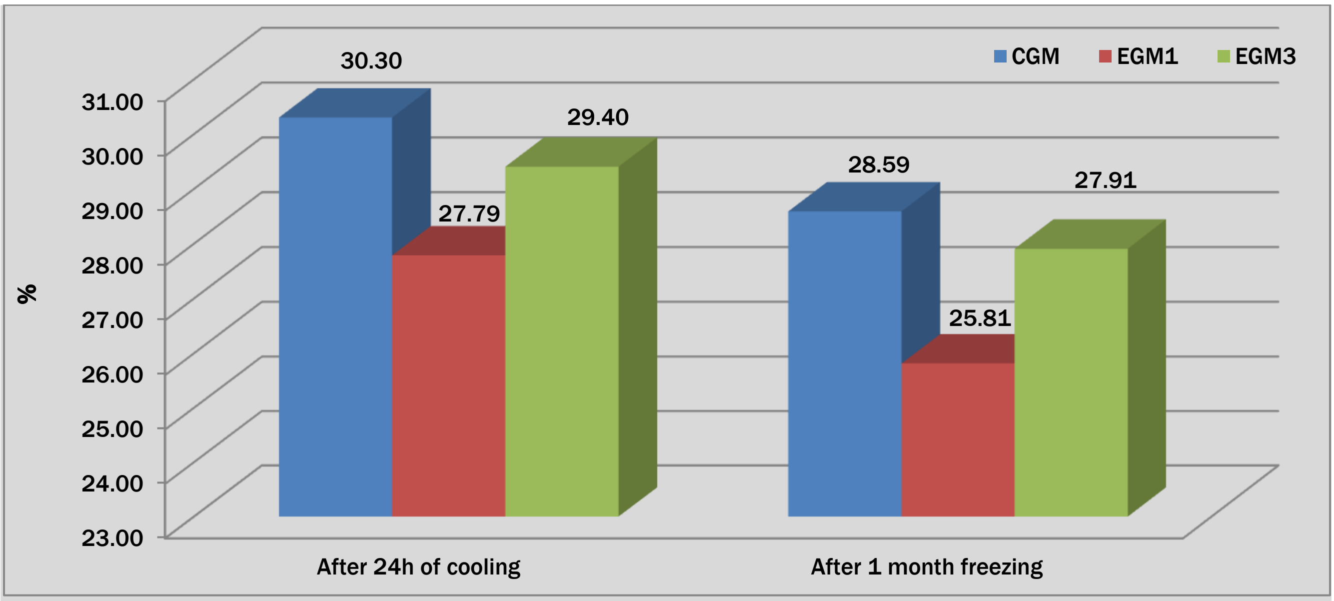

Figure 9 - Cooking loss before and after freezing of mutton (%).

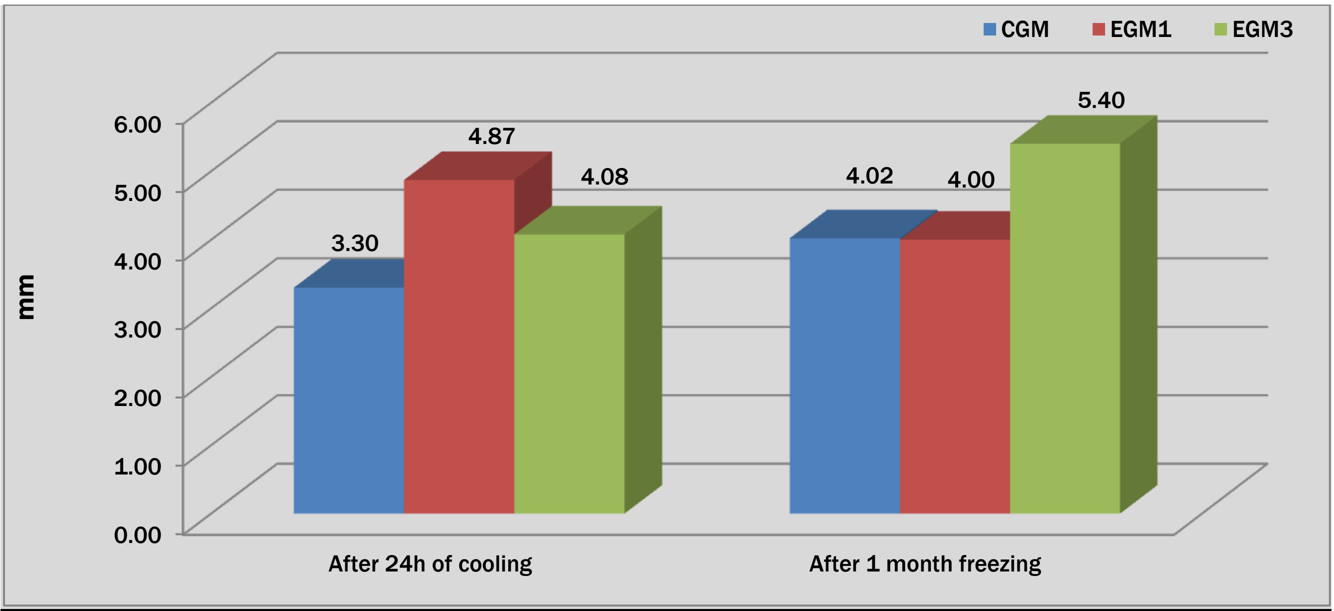

Figure 10 - Tenderness of mutton before and after freezing (mm). (Meat tenderness was negatively correlated (P<0.01) with b* results after 24 h of cooling and 1 month of freezing).

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CONCLUSION AND RECOMMENDATIONS

Mutton (Awassi), a common meat source for human consumption on the Lebanese market, is characterized by lesser fat that is concentrated in the fat tail, making it a healthier meat option for the Lebanese customer who prefers it above all other products of animal origin. In practical comparisons comparing the effects of Sage replacer to antioxidant in feed with varied percentages of 1 and 3 percent, there are few data from both local and global practical applications. Sage may have positive effects on Awassi sheep through stimulation of the immune system, stimulation of feed intake and digestive secretions, anti-bacterial, antiviral, or anti-inflammatory action, and antioxidant qualities when added in small doses to basic rations. In order to feed their herds rations laced with sage and other natural herbs as growth promoters without using synthetic antioxidants, Lebanese farmers must adopt a new way. To ascertain the true economic impact of utilizing sage on body performance and meat quality, we advise carrying out this practice in a commercial herd. Additionally, Awassi male sheep fed basic rations with sage supplements of varying concentrations (percent) should be used in future studies to determine the optimal concentration that improves animal performance. As a result of our findings, we advise using sage as a basic ration addition in Awassi sheep feeding in a proportion of 1% *S. officinalis*.

DECLARATIONS

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Conflict of interests

The author did not declare any conflict of interest.

Data availability statement

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

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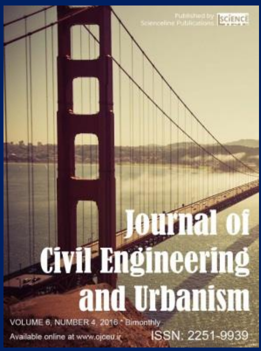

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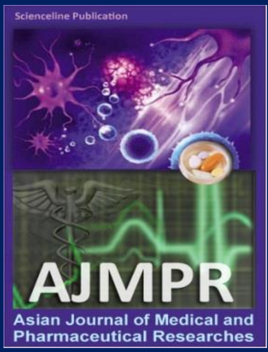

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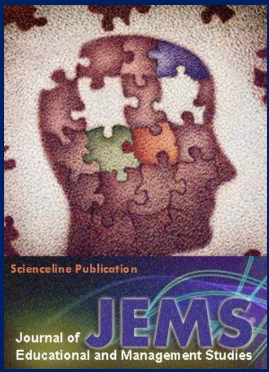

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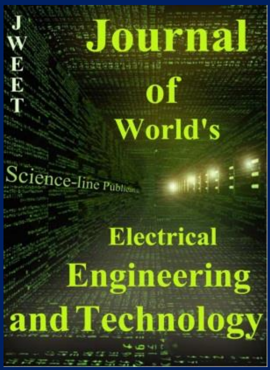

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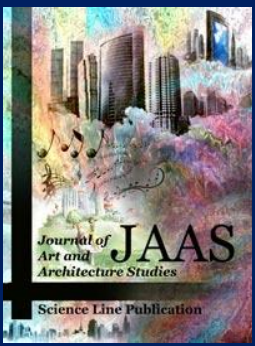

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